CHARACTERIZATION OF GELBSTOFFE IN MONTEREY BAY BY NYLON ADSORPTION, UV, AND PAPER CHROMATOGRAPHY

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THESIS

Characterization of Gelbstoffe in Monterey Bay
by
Nylon Adsorption, UV, and Paper Chromatography

by

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March 1972

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by

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Submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE IN OCEANOGRAPHY

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ABSTRACT

The dissolved organic components of marine waters were studied using an inverse flow glass filter system to separate the particulate and dissolved constituents. Cell rupture was prevented by the use of a gentle positive hydraulic head. Concentrations of humic acids were obtained by a nylon adsorption column which also desalted the sample. The process provided concentrations up to 10,000 times. Paper chromatography and ultra-violet spectrometry were used to characterize the dissolved humic content of water from Monterey Bay and offshore ocean stations. The correlations between sources indicated the relative importance of organic acids, amino acids, phenols, and carbohydrates in sewage outfalls, kelpbeds, harbor areas, river mouths, rip currents and industrial discharges.

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ACKNOWLEDGEMENTS

The author expresses his appreciation to:

Dr. Eugene D. Traganza for his critical review of this work and untiring assistance as thesis advisor.

Dr. Charles F. Rowell for his advice, assistance, and critical review of the manuscript.

The Chemistry Department at the Naval Postgraduate School and associated facilities, especially Ken Graham, for the use of equipment and knowledge available.

Miss Jeannie Keen and Miss Kathleen Byrne for their aid in preparing the manuscript.

Mr. Gene Bobo and the Chadbourn Hosiery Company for providing the nylon for the adsorption columns and advice on its use.

I. INTRODUCTION

605×10^9 tons of organic carbon are in solution in the oceans (Jeffrey, 1970). This enormous amount of organic material is four times the annual natural carbon production on the entire Earth. In spite of the bulk present, concentrations of dissolved organic matter are only of the order of 1 mg C/l above 600 meters and 0.5 mg C/l below 600 meters in the open ocean (Duursma, 1961, 1963) compared with 35,000 mg Salt/l. Concentrations increase in coastal waters.

By definition, organic material is dissolved if it can pass through a 0.45 micron filter, and if it does not precipitate out at pH 3.5. Low concentrations and interference from the relatively massive concentrations of salts are the major problems in studying dissolved organic matter. The difficulties in concentration, isolation, and desalting of dissolved organic matter in addition to false results caused by contamination of outside organic sources, has limited our understanding of their importance in oceanography. Paradoxically, much more is known about the smaller fraction of organic particulate matter (living and non-living) than the dissolved organic material, even though there is 10 to 100 times as much dissolved organic matter as particulate in the oceans.

Certain organic metabolites dissolved in sea water are responsible for the distribution and growth of algae, bacteria, protozoa, and various larvae (Lucas, 1955). Other organic compounds are essential to growth of the food chain (Wagner, 1969). Dissolved organic pollutants and toxins kill entire populations of marine life (Hood, et al.,

1960), and still other organics are important to certain sediment formations (Degens, et al., 1964). The organic molecules sorbed on suspended particles in the ocean eventually settle to the bottom and participate in the formation and accumulation of petroleum (Slowey, et al., 1962).

The Navy has become interested in the acquisition of chemical data as an aid in solving the problem of ambient noise in underwater acoustics. "Predictive models of ocean distribution of bioacoustic properties may be formulated from distribution of chemical organic and inorganic nutrients or inhibitors" (Traganza, 1971). The Navy is also involved in the monitoring of the optical properties of sea water which are a function of the amount of dissolved organics.

One of the most interesting early investigations into dissolved organic matter was that of Kalle (1933) who, working in coastal waters, attributed color changes in sea water to concentration variations of yellow humic acids, "Gelbstoffe." Later, Kalle (1960) characterized Gelbstoffe as being a combination of "phenolic-humic" acids and "nonphenolic carbohydrate-humic" acids, or "melanoidines." He experimented with many mechanisms for studying Gelbstoffe, especially optical properties of sea water samples as a function of salinity, but his results were "not very definite.....largely because of the complexity of the mixture of organic substances present" (Kalle, 1966). Degens (1966) and Craige and McLachlan (1964) suggested that the phenolic and amino acid constituents of sea water are the two organic groups dominant in the composition of humic substances or yellow discoloration known as "Gelbstoffe." These compounds evoke special interest since some, especially the phenolic constituents, have toxic

properties for bacteria (Conover and Sieburth, 1964), algae (McLachlan and Craige, 1964), and larval animal forms (Conover and Sieburth, 1964). Hence, "These substances may thus have a central position in the biological role of organic matter of inshore waters" (Jensen and Sieburth, 1968). Duursma (1966) carried this work one step further by describing a possible humification process to build up organic molecules and characterizing the end product by a melanin configuration (Fig. 47). Duursma also made an important contribution by listing many dissolved organic compounds found in the sea. The polymolecular nature of Gelbstoffe was confirmed by Chassemi and Christman (1968) who reported that the molecular weight varied between 700 and 10,000.

John Sieburth (1968) reported using a nylon column to extract 70% of the humic matter from bog water, river water, and shore sea water in Norway. After ultra-violet spectrum analysis and paper chromatographic separations, he concluded that the yellow material in some terrestrial and in his sea water samples appeared to be derived from polyphenols. In later work, Sieburth (1969) studied exudates from Norwegian seaweed in an attempt to study a definitive natural source of Gelbstoffe. He hypothesized that Gelbstoffe was synthesized in algae exudates by a mechanism involving the combination of simple phenols with proteinaceous and carbohydrate matter. Kalle (1966) suggests synthesis of the nonphenolic fraction melanoidine from sugars and amino acids by microorganisms in the open ocean.

Jeffrey (1969) did extensive research tying together previous knowledge and methods for study of dissolved organics. In her doctoral dissertation in which she presents an excellent characterization of the organic nature of sea water off the Texas coast, she is impressed

by the possibility that humic acids are the dominate organic form, even in the open deep ocean. Wagner (1969) published another review of the individual chemicals and types of compounds identified, isolated, or reported present in sea water. In his study, Wagner also reported several procedures presently being used for dissolved organic analysis. In a similar but more extensive study, Diehl (1971) prepared a list of all known methods of dissolved organic analysis and proposed optimum systems for the sampling, desalting, concentration isolation, and identification of the different organic compound classes present in sea water.

With this background knowledge, the work described in this thesis was begun utilizing a promising new method which combined nylon columns with paper chromatography and ultra-violet spectrophotometry, and was a relatively simple though potentially powerful approach to the difficult and important study of "trace organics" in the ocean. The major portion of the study was focused on the humic acid nature of the coastal waters of Monterey Bay and the accompanying deep ocean. The deep ocean sample should represent the most refractory compounds which have the greatest probability of an "organic tag" for a specific water mass. The different sources of organics were characterized by their humic-acid content, by ultra-violet absorption spectra, and by R_f values on paper chromatograms, the hypothesis being that a possible correlation between the different stations would provide an empirical technique for study and characterization of water masses and possibly yield preliminary insight to the humic acid cycle for the Bay area. Such studies were advised by Kalle (1966) and Sieburth (1968) who felt more detailed research into the presence of humis would provide the

knowledge necessary for solving many dissolved organic matter problems existing today. Chromatograms of different water masses may become a sensitive tool for the study of sinks and sources of dissolved organic matter which can lead to a better understanding of marine processes. Finally, in addition to specific interest in dissolving organics, there are researchers who logically argue that we must determine the natural background of dissolved organics before the oceans become too polluted (Jeffrey, 1971).

II. METHODS

A. CONTAMINATION PREVENTION

A great amount of time and trouble was taken to prevent outside sources from contaminating the water samples. There were six general rules followed to insure a contamination-free system:

1. No polyethylene, rubber, cork, or organic compound was used as a bottle, sampler, stopper, or allowed to come in contact with the sample. This procedure also prevents the use of stopcock grease and glycerin.

2. A closed system was used to prevent the contamination in the air from producing prejudiced results. By a closed system, it is meant that at no time is the sample exposed to the open air any longer than necessary. A minimal exposure to air occurred at three times in the process: a) when the sample was collected; b) when the sample was filtered into a second carboy; and c) when the concentrated sample was collected in the 5 ml aliquots.

3. All glassware was washed as described below immediately before use.

   a. Scrubbed well with biodegradable soap and water.

   b. Rinsed in water and then in chromic acid cleaning solution (chromic acid and sulfuric acid).

   c. Rinsed in water and then in acetone, chloroform, and alcohol to remove any insoluble grease.

   d. Rinsed in organic free water (5 gm/liter Hydrogen Persulfate in distilled water).

e. Rinsed in distilled water.

f. Covered with aluminum foil.

4. Immediately after the sample was obtained and placed in the 20 liter carboy, the carboy was inserted inside two plastic bags. The temperature of the water sample was kept at its "in situ" temperature until after filtration by surrounding the carboy with cold water. This procedure was followed to prevent the contamination of dissolved organic material by the death of living organisms in the sample.

5. The time from obtaining the sample until the filtration process was complete was not allowed to be longer than 6 hours. This measure prevented the contamination by organism death and physiological processes which would naturally occur.

6. The determination of the optimum filtration system minimized contamination during the separation of the particulate and dissolved organic material from cell breakage.

The experimental controls were designed to check the degree to which contamination prevention was achieved.

B. SEA WATER SAMPLING

Water samples were obtained from eight stations in the Monterey Bay and from two stations in deep water 100 miles outside the bay (see Table I and Figure 1). These stations were chosen for their probable role in the dissolved organic system of this area with the exception of the offshore stations which were taken as controls. The surface water samples were taken in 20 liter carboys which were lowered just below the surface before their stoppers were removed. The midwater and deep-water samples were obtained by connecting ten Nansen bottles as closely as possible, and the fifth bottle lowered to the required depth. The

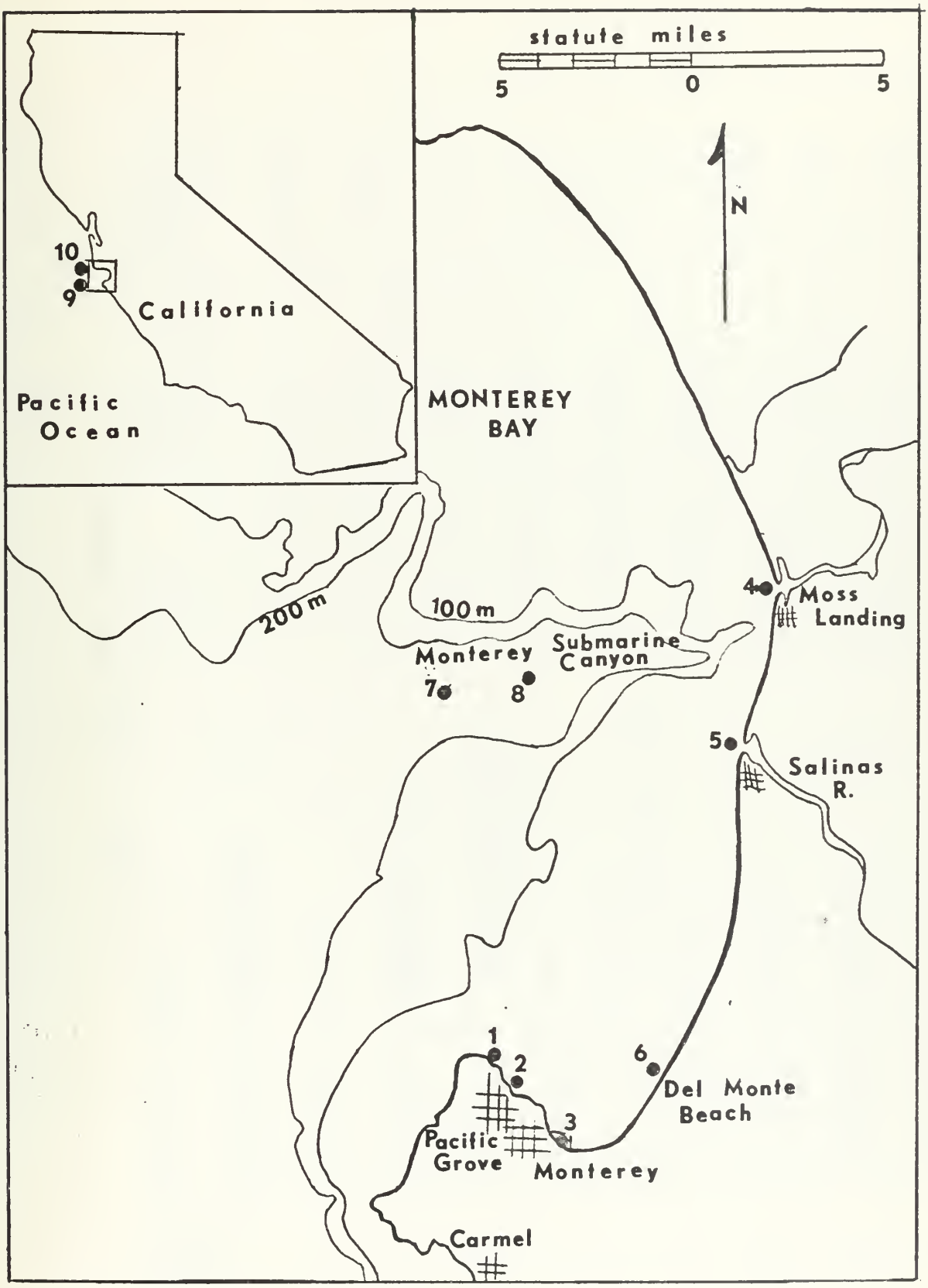


Fig.1. Map of sampling stations.

TABLE I: WATER SAMPLE STATIONS

Station Number	Position	Type	Date of Sample	Sample Depth(meters)	Sample Temperature
1.	121°55.9'W 36°38.3'N	Sewage Outfall (Pacific Grove, Calif.)	13 Jan. 1971	surface	18.3°C
2.	121°54.8'W 36°37.6'N	Kelp Beds	3 Apr. 1971	surface	11.8°C
3.	121°53.3'W 36°36.3'N	Marine Harbor (Monterey, Calif.)	21 Feb. 1971	surface	12.3°C
4.	121°47.5'W 36°48.3'N	Industrial Discharge (Moss Landing, Calif.)	20 Apr. 1971	surface	12.1°C
5.	121°46.7'W 36°44.8'N	River (Salinas River)	14 Mar. 1971	surface	11.6°C
6.	121°50.6'W 36°37.7'N	Rip Current (Del Monte Beach, Calif.)	20 Mar. 1971	surface	11.4°C
7.	121°55.0'W 36°47.5'N	Mid-Water	2 May 1971	100 m.	8.3°C
8.	121°51.1'W 36°48.0'N	Mid-Water	2 May 1971	100 m.	7.6°C
9.	123°45.2'W 35°57.1'N	Deep Water	27 Feb. 1971	1000 m.	4.7°C
10.	123°19.3'W 36°06.1'N	Deep Water	28 Feb. 1971	500 m.	5.1°C

samples were kept in 20 liter glass carboys.

C. FILTRATION AND CENTRIFUGATION

The purpose of the filtration and centrifugation was to remove the particulate matter greater than 0.45 microns suspended in the water sample. This material consisted of living and nonliving particulate. Any system for separation must meet three criteria. First, it must not destroy or break down the particulate matter in any way, which might add to the dissolved organic content of the sample. Second, the system must have a process rate rapid enough to prevent natural chemical reaction or decomposition of particulate material from altering the original organic content of the sample. And third, the system must not in itself change the sample's organic content. The experiments described below were run to determine the optimum method for separation of particulate matter.

1. Procedure I (Filtration)

This experiment was an all-filtration method in which a series of four glass filters of decreasing pore size were used for separation. The filtration battery consisted of a coarse filter (40-60 microns), a medium filter (10-15 microns), a fine filter (4-5.5 microns), and a final filter (0.46 microns). The final filter was a Millipore filter with interchangeable glass filters. The filters were arranged sequentially in a horizontal plane with the flow being up through each filter instead of down (see Figures 2 and 3). The filter system was designed so as to prevent any one of the filters from becoming clogged. The configuration provided low points where particulate material could collect away from the filter surfaces. A gentle hydraulic head was

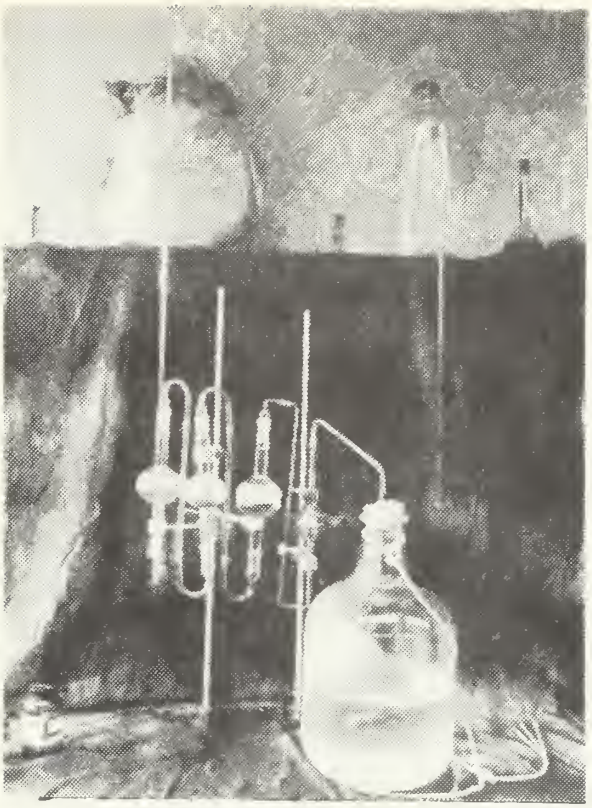


Fig.2. Inverse flow glass filtration system for the separation of particulate matter from sea water using a gentle hydraulic head.

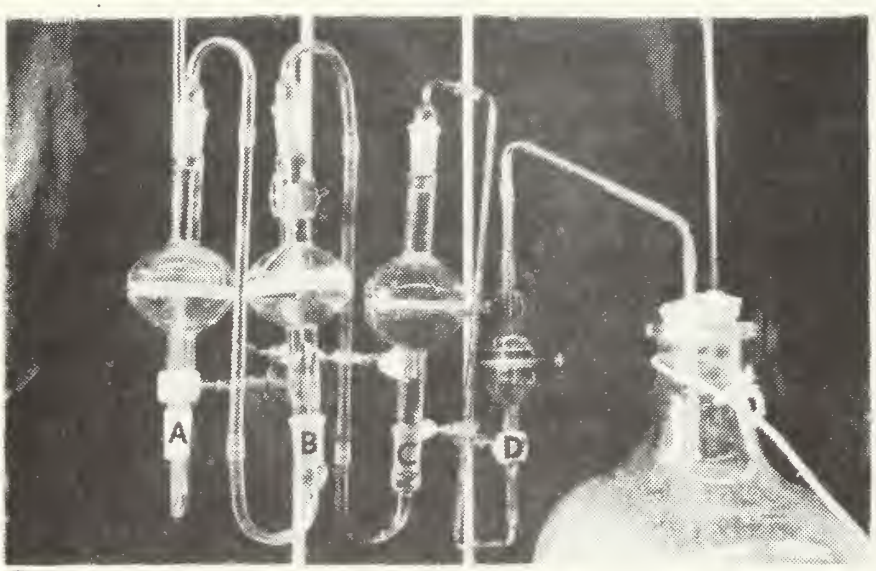


Fig.3. Inverse flow glass filter battery: (A) coarse filter (40-60 microns), (B) medium filter (10-15 microns), (C) fine filter (4-5.5 microns), and (D) final Millipore filter (0.46 microns).

used to produce the flow in place of the more violent vacuum (negative pressure) method.

Experiment 1a was run to determine the optimum height of the water sample level above the filters. This height would produce the least amount of dissolved organic alteration balanced with the fastest flow rate.

a. Experiment 1a

A source of concentrated living particulate material was obtained by a series of vertical plankton tows at the end of Wharf II of Monterey Harbor. The plankton was kept in natural sea water with the temperature adjusted to that of the sea surface to minimize death due to change of environment.

25 ml of the plankton concentrate was placed in 1 liter of temperature controlled sea water, and the sample level elevated to a height above the filter.

After 50 ml of the sample had flowed up through the filter, the sample was removed.

1 ml of the filtrate and 1 ml of sample from the U-shaped reservoir under the filter were examined on a counting tray under a microscope. The broken cells in 8 out of a total of 40 blocks were counted and recorded from each of the 1 ml samples.

For the same filter, the process was repeated for hydraulic heights of 1, 2, 3 and 4 feet. The series of four tests had to be done from the same plankton solution which was kept cool to minimize variations in plankton content. It was assumed that the plankton concentration was homogenous throughout the plankton solution.

The experimental steps were run twice with the hydraulic heights being adjusted in the order 1, 2, 3 and 4 feet, and then the experiment was repeated twice with the heights adjusted 4, 3, 2 and 1 feet. In this way, the order of height adjustment was not a major experimental factor.

The above procedure was followed for each of the four filters and the results were averaged for each. The filtrate broken cell count and the reservoir broken cell count were graphed against hydraulic height (Fig. 11-14) for each filter. The only variation in procedure was to omit the filtrate from the final filter, whose filtrate was not examined since it contained dissolved material only. A different test was run; the direction of flow was reversed on the filter so as to release trapped particulate matter, and 10 ml of reverse filtrate was collected. 1 ml of this filtrate was examined and the broken cell count determined and graphed as directed above (Fig. 11-14). These results indicated the relationship between height and cell destruction.

b. Experiment 1b

A 1 liter sample from Del Monte Beach was placed at a determined height above one of the filters.

After a constant flow rate was obtained, the filtrate was collected at 1 minute intervals for exactly 10 minutes, and the volume recorded in ml.

The flow rate at the hydraulic heights of 1, 2, 3 and 4 feet was measured three times for each filter.

The average flow rate (ml/sec) was plotted against hydraulic height (Fig. 11-14). These results indicated the relationships between height and flow rate. A study of these graphs indicated the

optimum hydraulic height.

2. Procedure II (Centrifugation)

The second experiment investigated a combination of centrifugation and filtration in order to decrease the process time without increasing the dissolved organic alteration. Experiments were run in order to obtain the optimum centrifuge time and rate. Then the optimum filtration rate was determined in order to ensure minimum process time.

a. Experiment 2a

A plankton tow was made and preserved as in Experiment I.

50 ml of the plankton was diluted with 800 ml of temperature-controlled sea water. The plankton was then poured into the seven 100 ml centrifuge bottles of the centrifuge. The No. 7342 Precision Scientific Co. centrifuge has three speeds: 600, 1200, and 1800 rotations per minute.

Four of the bottles were placed in the centrifuge and centrifugation begun at 600 RPM. One bottle was removed at 5, 10, 15 and 20 minute intervals and a bottle of equal weight put in its place to counterbalance the rotation.

As each bottle was removed, 1 ml of suspended solution was put on a counting tray and the suspended cells were counted as in Experiment I. Then 1 ml of solution sediment was obtained from the bottom of the bottle and the broken cells were counted. The results were recorded. The procedure was repeated twice more and the results of the three tests averaged.

The suspended cell count and the sediment broken cell count were plotted against time (Fig. 15-17).

The entire procedure was repeated for 1200 and 1800 RPM. From this experiment, an optimum centrifuge time was determined for each of the three speeds.

b. Experiment 2b

Plankton was obtained and diluted as in Experiment II - 1a. The temperature of the plankton solution, not in use, was kept constant.

A bottle of plankton solution was spun at each of the three centrifuge rates for the corresponding optimum time as determined in Experiment 2a.

Suspended cell count and sediment broken cell count were determined as in Experiment 2a and the results plotted against RPM. The final result is the optimum centrifuge rate and time (Fig. 18).

Experiment 3 was run to determine the flow rate characteristics of the filtration system. Tap water was adjusted to a salinity of 35% with NaCl to approximate the centrifuged sample.

3. Experiment 3

20 liters of the artificial sea water was prepared for use in the experiment. The filters were numbered 1, 2, 3 and 4 in order of decreasing pore size.

The filters were arranged in all possible combinations without placing a smaller pore filter in front of a larger, and with always placing filter 4 at the end of the filter battery. These combinations were 1, 2, 3, 4; 2, 3, 4; 1, 3, 4; 1, 2, 4, 1, 4; 2, 4; 3, 4; and 4.

For each combination, a constant flow rate was obtained, and then when the hydraulic head was the optimum as determined in Experiment I, the flow rate was determined over a 10 minute period. The experiment was rerun twice to ensure correspondence of results, and

filter systems were plotted against flow rate (Fig. 19).

4. Experiment 4

The last experiment in the determination of the best centrifugation/filtration method was a chemical comparison of Procedures I and II.

A homogenous plankton sample, containing 100 ml of plankton diluted to 2 liters of sea water was divided in two; 1 liter of the sample was processed by Method I and 1 liter by Method II. Procedure II was prejudiced to the extent that only 500 ml of sample could be centrifuged at one time.

A total process time for each method was recorded and reported in the results. An ultra-violet spectrum and a paper chromatogram were run from the concentrated dissolved organic material obtained by each method as a means of recording differences. The process was repeated twice to get some replication.

5. Sample Processing

In the absence of a large volume centrifuge, all the water samples from the ten experimental stations were processed by Procedure I. During the entire filtration process, 20 liter carboys, containing the water samples, were kept inside plastic bags that were placed around them after the samples were taken. The temperatures of the samples were continuously monitored to minimize death of the organism. Each sample was run up through the series of filters. The height of the carboy above the filters was adjusted periodically in order to maintain the optimum flow rate.

It was usually necessary to change the 0.45 micron glass filter once every 15 minutes for samples with heavy organic concentrations and

once every 30 minutes for samples with light organic concentrations. The change was arbitrarily determined by the color of the sample which was a function of the organic concentration (Riley, 1965). Hence, the clearer the sample, the fewer the times the filter had to be changed. Total sampling time was on the average about 4 hours for each sample.

Once the sample had been filtered, the pH was adjusted to 3.5 and the sample run back through the final filter. This final process is in accordance with Riley's (1965) definition of dissolved organics which specifies that organics will not precipitate out of solution at pH 3.5. According to Sieburth (1968), the acidic solution also helped prevent chemical alterations of the dissolved organic material. The water sample was then ready to be placed on the nylon column.

D. ADSORBENT COLUMNS

1. Preparation of Nylon Columns

The adsorbent material used in the adsorption columns was an undyed crepe nylon (Chadbourn Hosiery, Charlotte, North Carolina). To remove impurities, the nylon was soaked in one liter of distilled water at 60°C containing a mixture of 5 grams caustic soda and 5 grams soap. The solution bath was brought to a boil and 100 ml of 10% sodium hydrosulphite was added. A slow boil was maintained for 15 minutes.

The nylon was removed, rinsed in cold distilled water, followed by a rinse in distilled water at 60°C for 15 minutes or until cleaned of detergent (Bobo, 1971).

The nylon, which was kept wet to prevent the intrusion of air, was tightly rolled and carefully packed into a 200 ml glass column (see Fig. 5). The absence of air channels is necessary for the optimum

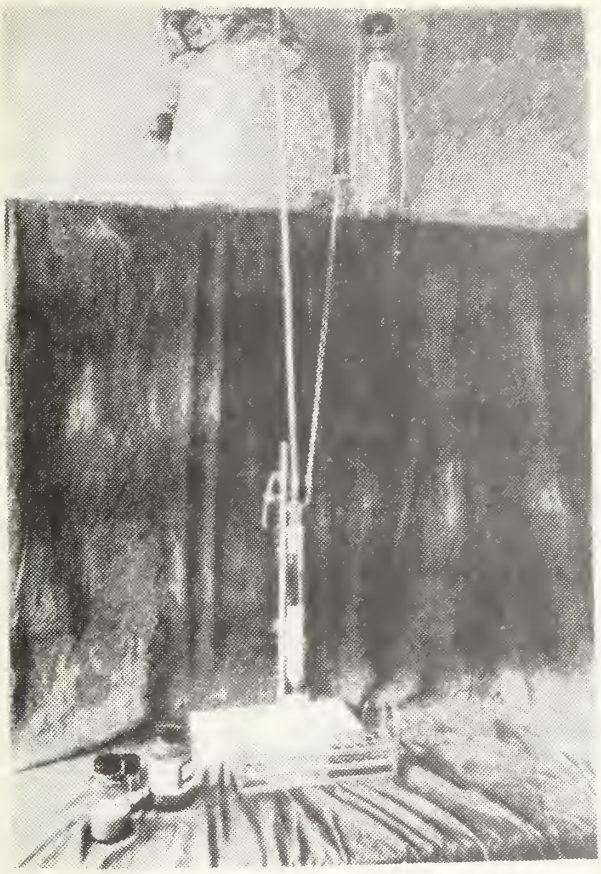


Fig.4. Nylon adsorbent column for the concentration and desalting of dissolved organic material in sea water.

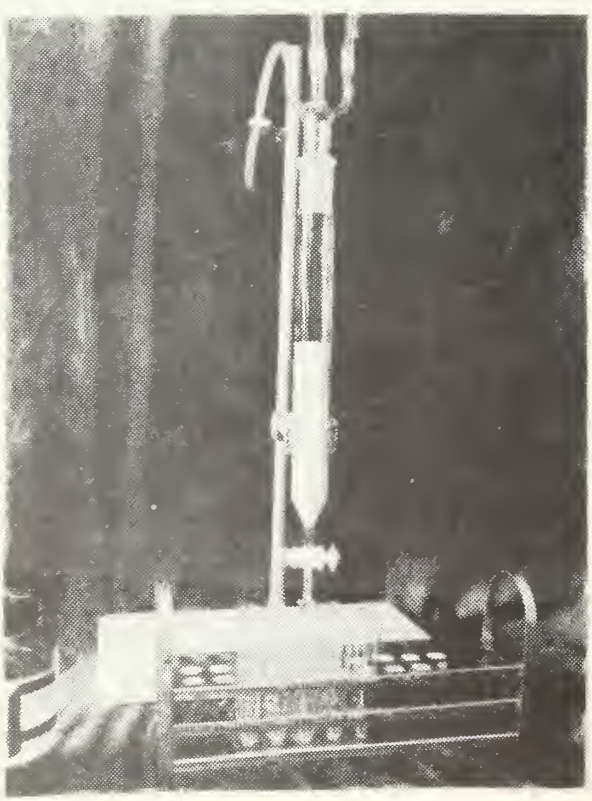


Fig.5. Nylon Adsorbent Column (close up).

efficiency of the column. Distilled water was run through the column until a constant flow rate was achieved, and then 20 ml of .1N NaOH was added as a final wash against contaminating compounds. A rinse with distilled water removed the NaOH.

2. Processing Samples

Once the effluent was neutral again, the acidified sea water sample was passed through the column (see Fig. 4). After the sample, approximately 200 ml of distilled water was used to flush the nylon of inorganic salts and non-adsorbed organic matter. The adsorbed compounds were then eluted with 20 ml of .1N NaOH, and brought off the column with distilled water. The concentrated organic material was collected in 5 ml fractions. The presence of phenolic and amino compounds was tested in each of the fractions by colorimetric tests. Two or three drops of each fraction were tested with ninhydrin reagent (2% by weight of 1, 2, 3-triketohydrindine in 50 ml ethanol, 100 ml H_2O, and 850 ml n-butanol) for amino compounds, and 2% ferric chloride solution for phenols. The 5th through 15th fractions after the elutant became basic were usually found to contain the desired compounds. The concentrated sample was neutralized and stored in refrigeration at $5^{o}C$.

E. CONCENTRATION BY VACUUM EVAPORATION

Final concentration of the sample was accomplished by a vacuum evaporator (Fig. 6). One side of the evaporator containing the sample was submerged in a mineral oil bath, maintained at $35^{o}C$. The other side was submerged in an ice bath, and a vacuum was achieved in the system by a water aspirator. The vacuum evaporation of the water was continued until the organics were concentrated in about 10 ml of solu-

Fig.6. Vacuum evaporation apparatus for the reduction of the sample volume: (A) 35°C oil bath containing glass bulb for sample, (B) ice bath containing glass bulb for evaporated solvent, and (C) vacuum aspirator safety bottle.

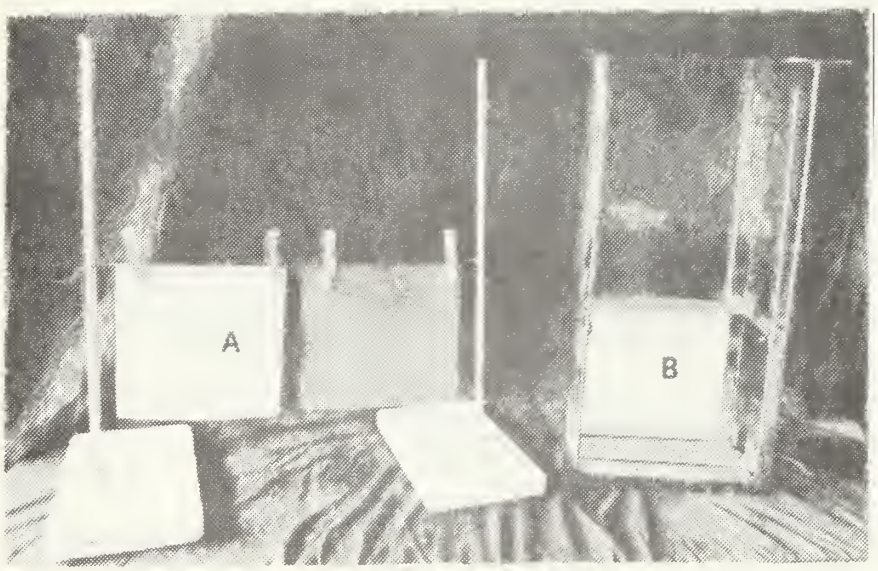


Fig.7. Ascending paper chromatography apparatus: (A) drying paper chromatograms, and (B) chromatography chamber with developing paper chromatograms.

tion. Hence, from a beginning volume of 10 liters, the organics were concentrated an additional 1000 times over the column concentration.

F. EXPERIMENTAL CONTROLS

As a check for contamination from experimental procedures or from apparatus, controls were run after each sample in order to duplicate the method followed except that distilled water was used. In the case of surface samples, 10 liters of distilled water was placed in a 20 liter carboy and processed. In the case of mid or deep water samples, the distilled water was placed in freshly washed Nansen bottles before being run through the experimental procedure. A paper chromatograph and an ultra-violet spectrum were used to detect the presence of contaminating chemicals.

G. PAPER CHROMATOGRAMS

The paper chromatograms of the concentrated organic material from the water samples were run on 20 x 20 cm sheets of No. 1 chromatographic filter paper (W & R Balston, Ltd, England). The sheets were first developed in distilled water and dried in an oven at 80°C in order to reduce interferences from fluorescent and reagent-staining nitrogeneous and phenolic impurities. About 25 ml of the sample was spotted on the paper with a microsyringe in position for a two dimensional chromatogram (a corner of the paper) and the sheet developed in the first direction with 15 ml of distilled water. After the chromatogram was fully developed, it was dried and developed in the second direction with 15 ml of acetic acid - ethyl acetate - water (2:6:2 by volume). Every chromatogram was run in quadruplicate and immediately after drying from the second phase, was marked under ultra-violet light for

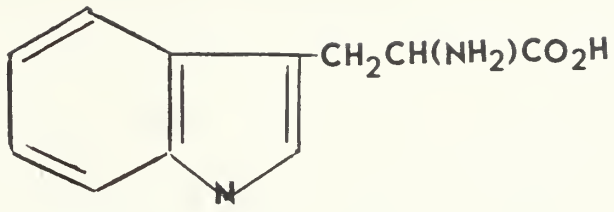


Fig. 8. L-Tryptophan

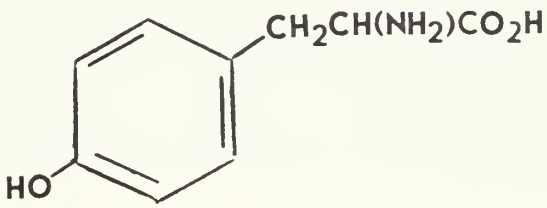


Fig. 9. L-Tyrosine

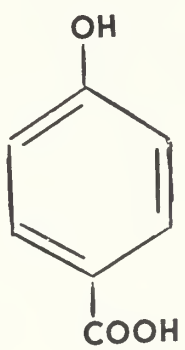


Fig. 10. p-Hydroxybenzoic Acid

fluorescent spots. Two chromatograms were sprayed with a detection reagent of ferric chloride-potassium ferricyanide consisting of 1 part of a 15% $FeCl_3$ and 1 part of a 1% potassium ferricyanide solution, and while still wet, the chromatogram was washed with dilute HCl. A blue reaction indicated the presence of reducing compounds and phenols. The other chromatograms were sprayed with a standard ninhydrin reagent (2% by weight of 1, 2, 3-triketohydrindine in 50 ml ethanol, 100 ml water, and 850 ml n-butanol). A purple color indicated the presence of amino acids (Hais and Macek, 1967) (see Fig. 7).

An R_f value was determined for each spotted compound on the chromatogram. R_f is defined as the distance the substance travels from the origin divided by the distance the solvent front travels from the origin. In addition, the position of each compound was recorded using a grid system with the chromatogram being divided into 49 (7 by 7) equal squares.

When a chromatographic spot developed in a block of the grid, it was labeled: a letter (P, Pf, A, Af, or f) for the chemical nature of the compound (phenolic, fluorescent phenol, amino, fluorescent amino, or fluorescent) and a set of grid numbers (numer of row - number of column). If the spot developed in more than one block of the grid, each block number was listed separately on the chromatogram (see Fig. 20). For better analysis, these different sets of grid numbers were consolidated into one set listing row numbers separated by commas, a dash, and then the grid numbers for the columns separated by commas.

H. ULTRA-VIOLET SPECTRA

The ultra-violet spectra of the water samples, experimental con-

trols, organic standards, and concentrated organics from the methods experiment were determined with a Beckman DB spectrophotometer. An incandescent lamp was used between 450 and 320 millimicrons (mμ), and a hydrogen arc lamp was used between 320 and 200 millimicrons. The spectra were run with the spectrophotometer on "narrow" slit recording at 40 mμ/min. The spectra were recorded as percent transmission (%T) versus wavelength (mμ).

I. DISSOLVED ORGANIC CONTENT

The dissolved organic content selectively collected by the nylon column sample was estimated quantitatively by weight for each sample. After the dissolved organics were removed from the column and neutralized, they were placed in a 100 ml flask and diluted to 100 ml total volume with distilled water. The weight of the solution and flask was compared with the weight of the flask containing distilled water and an equal volume of HCl and NaOH. The difference in weight milligrams (mg) divided by the total volume of the sample in liters was recorded as milligrams "C" per liter sea water or mgC/l.

J. ORGANIC STANDARDS

Seven organic standards were chosen for comparison with sea water samples: L-homogentisic, L-phenylalanine, vanillic acid, syringic acid, L-tyrosine, L-tryptophan and p-hydroxybenzoic acid. Unfortunately, only the latter three were available. The standards were chosen because of their presence in the ocean and their theorized importance to humic compounds as reported by Undenfriend (1962) and Riley (1965). Paper chromatograms, UV-spectra, and dissolved organic content were determined for each compound.

In addition, the efficiency of the absorption column was measured by using the standards. A known concentration of standards was placed on the nylon column, and compared with the concentration recovered using the Beckman DB, and Beer's Law theory for correlation of absorbance and concentration. The percent recovery of each standard was determined in this manner using the absorbance of the $FeCl_3$ derivative for p-hydroxybenzoic acid and a ninhydrin derivative for L-tryptophan, and the neutral absorbance of L-tyrosine.

III. RESULTS

A. FILTRATION AND CENTRIFUGATION

The graphs (Fig. 11-14) from the filtration experiment indicated that each filter had a different optimum height, ranging from a maximum of 2.95 feet for the coarse filter to 1.84 feet for the final filter. The hydraulic height of 2.25 feet was chosen as the optimum height for the system.

The results which are graphed in Figures 15-17 indicated that each centrifugation speed had in optimum time, but as one can see in Fig. 18 the slowest RPM, 600, at its optimum time was the best centrifuge procedure. Not all of the particulate material can be separated by centrifugation without cell rupture and a final filtration is necessary to isolate only the naturally occurring organic chemical species. The final filter alone provided the fastest flow rate (Fig. 19).

Comparisons of Procedure I and II recorded and tabulated in Fig. 24 and Table III. The determining factor for the superior procedure is the difference in process time after all rupture is minimized. The centrifugation-filtration procedure was 1.3 times faster than the filtration method alone.

B. PAPER CHROMATOGRAMS

The chromatographic controls and spectroscopic analysis of the experimental controls permitted a contamination characterization of the system. The glass filter-nylon column system was free of fluorescent (Fig. 20) and UV-absorbing (Fig. 38) contaminates, but contained

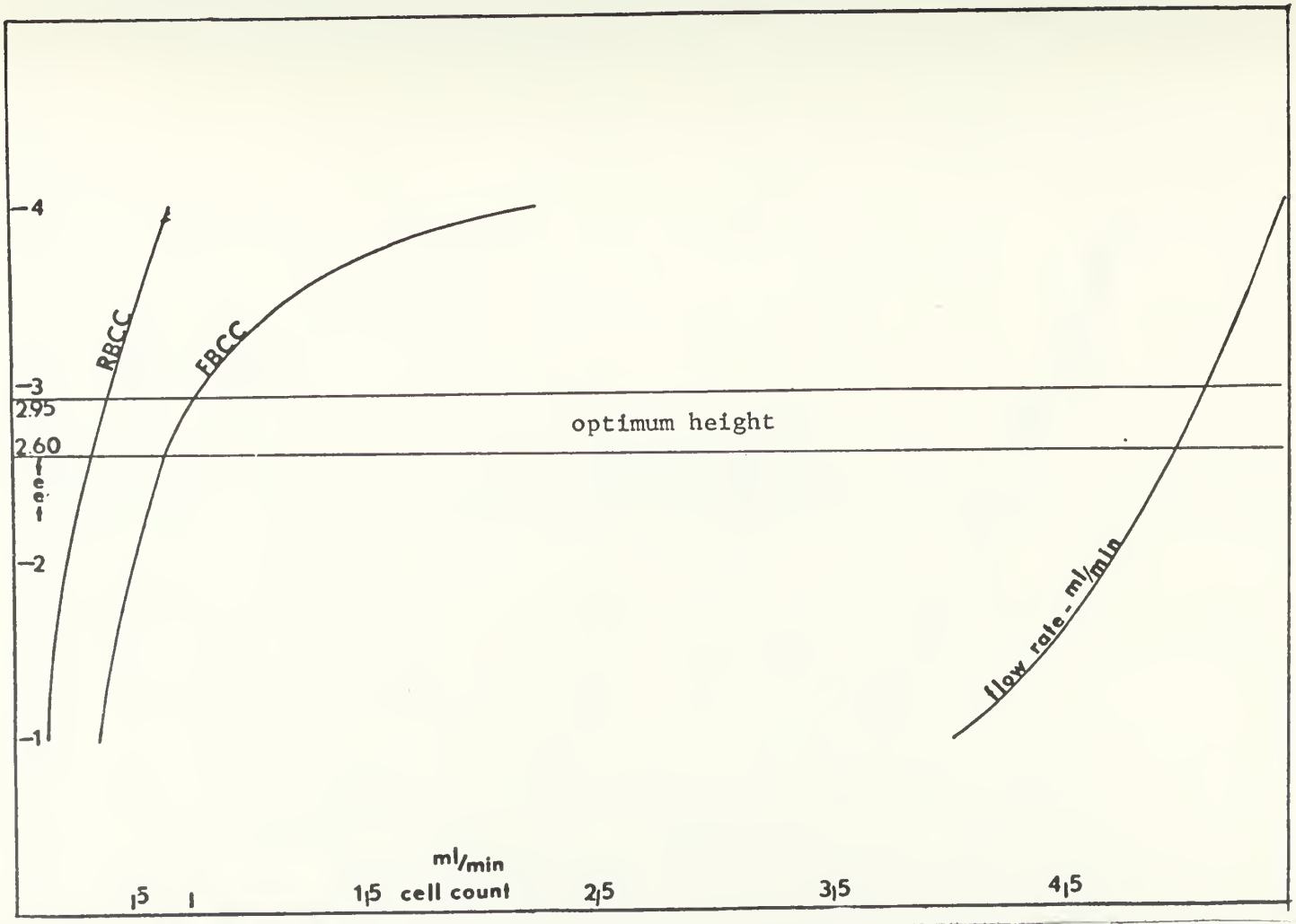


Fig. 11. Optimum filtration height for coarse filter (40-60 microns): filtrate broken cell count (FBCC), reservoir broken cell count (RBCC), and flow rate (ml/min) versus hydraulic height (feet).

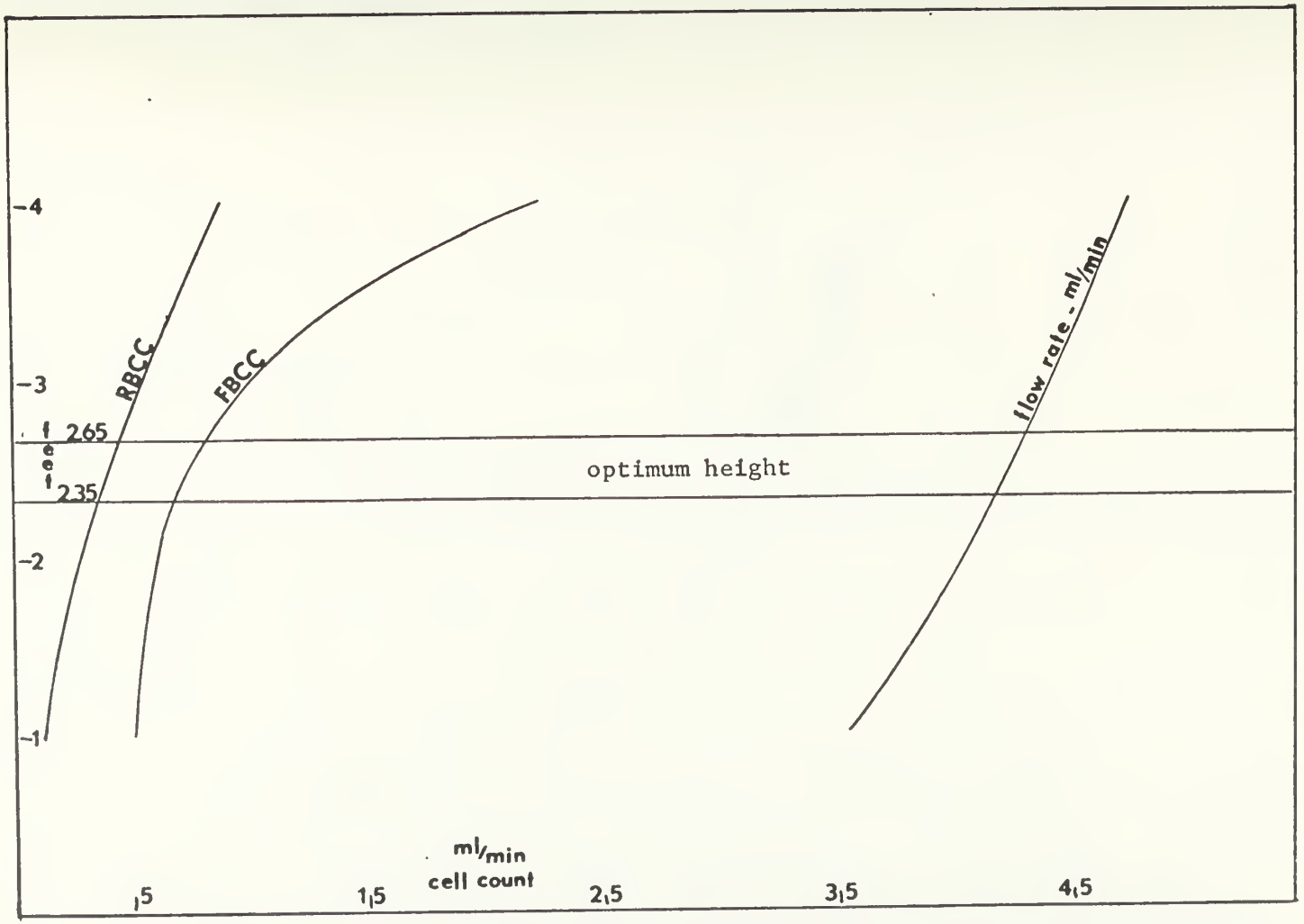


Fig. 12. Optimum filtration height for medium filter (10-15 microns): filtrate broken cell count (FBCC), reservoir broken cell count (RBCC), and flow rate (ml/min) versus hydraulic height (feet).

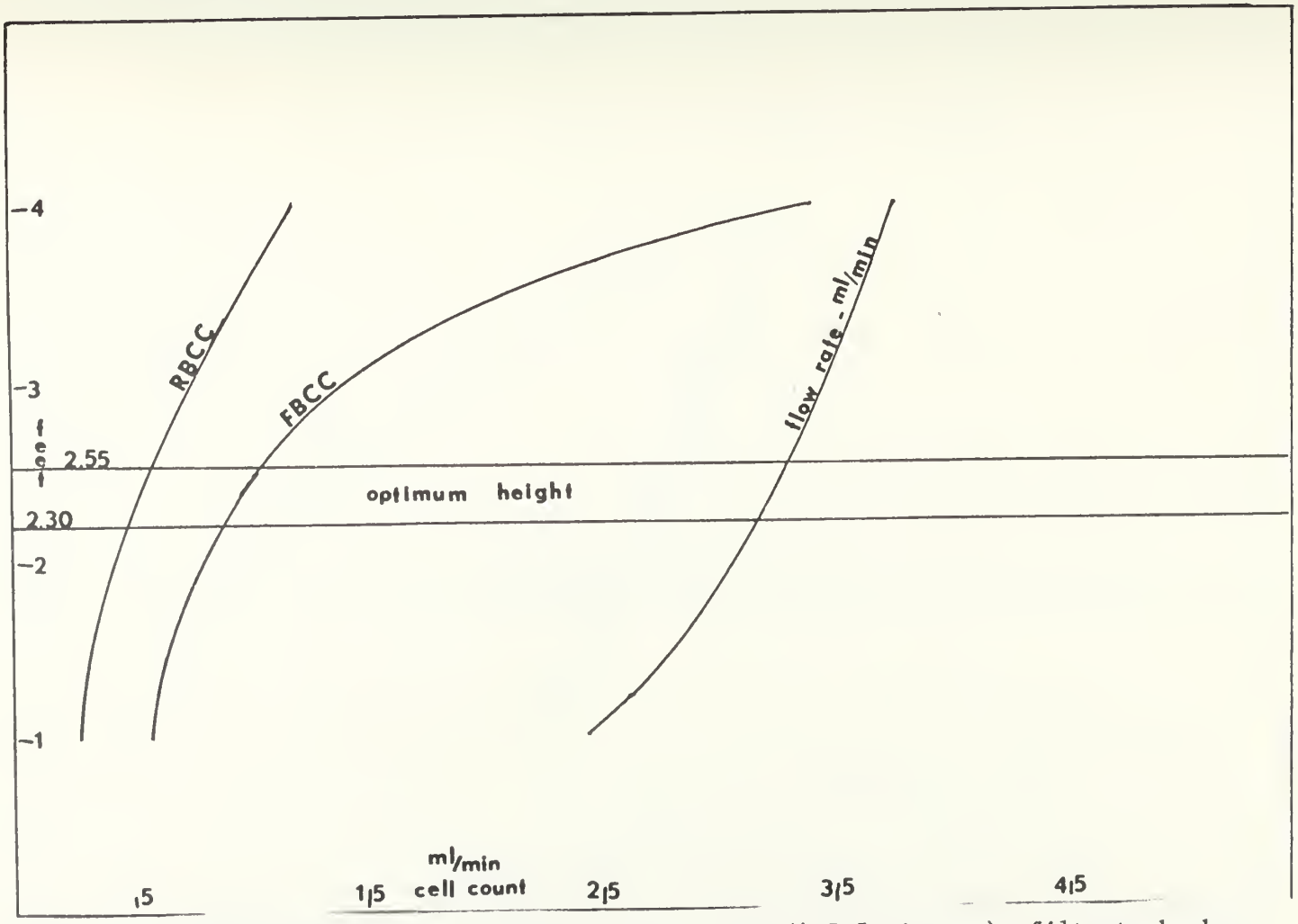


Fig. 13. Optimum filtration height for fine filter (4-5.5 microns): filtrate broken cell count (FBCC), reservoir broken cell count (RBCC), and flow rate (ml/min) versus hydraulic height (feet).

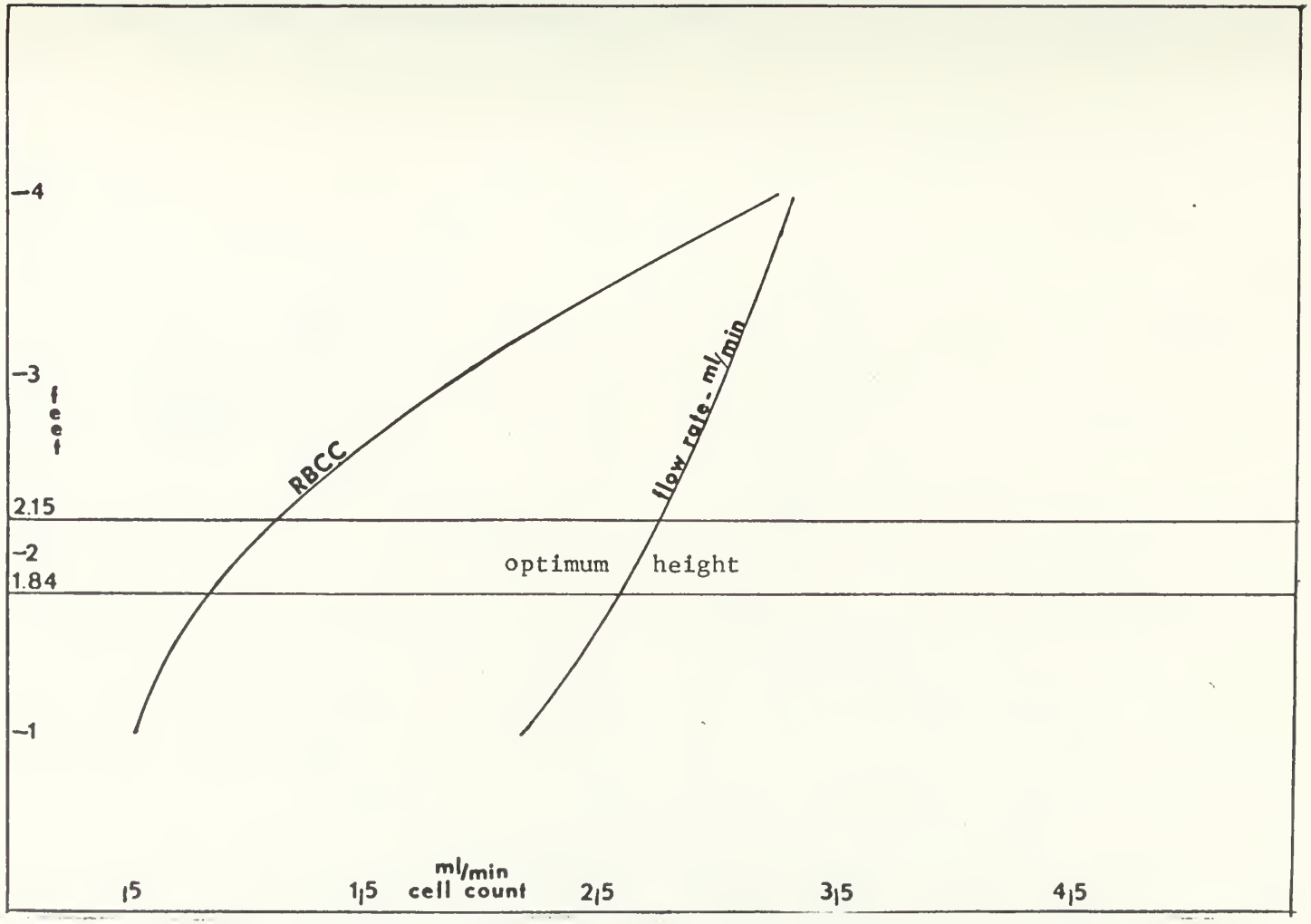


Fig. 14. Optimum filtration height for final Millipore filter (0.45 microns): reservoir broken cell count (RBCC) and flow rate (ml/min) versus hydraulic height (feet).

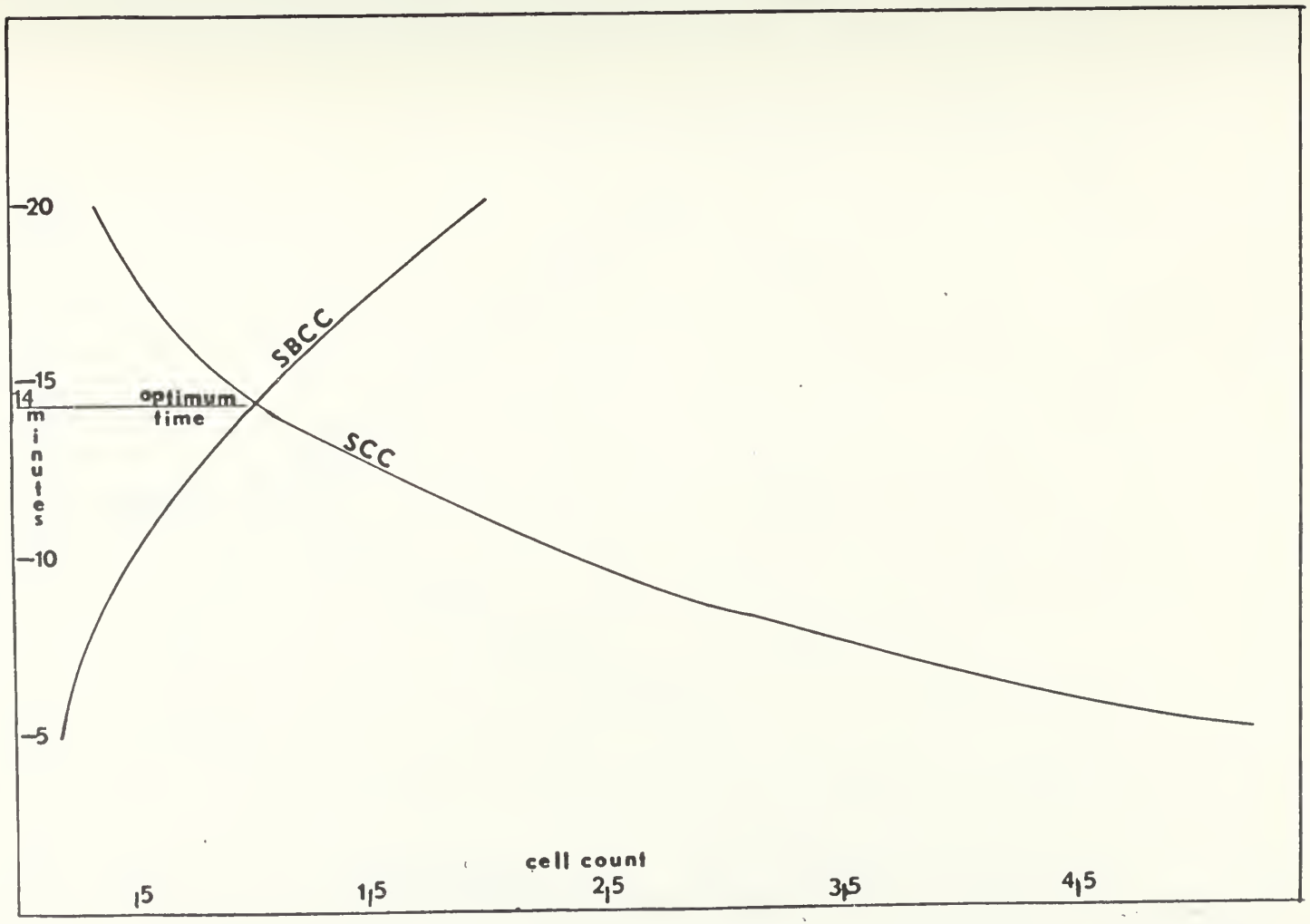


Fig. 15. Optimum centrifugation time for 600 RPM: suspended cell count (SCC) and sediment broken cell count (SBCC) versus length of centrifugation (min).

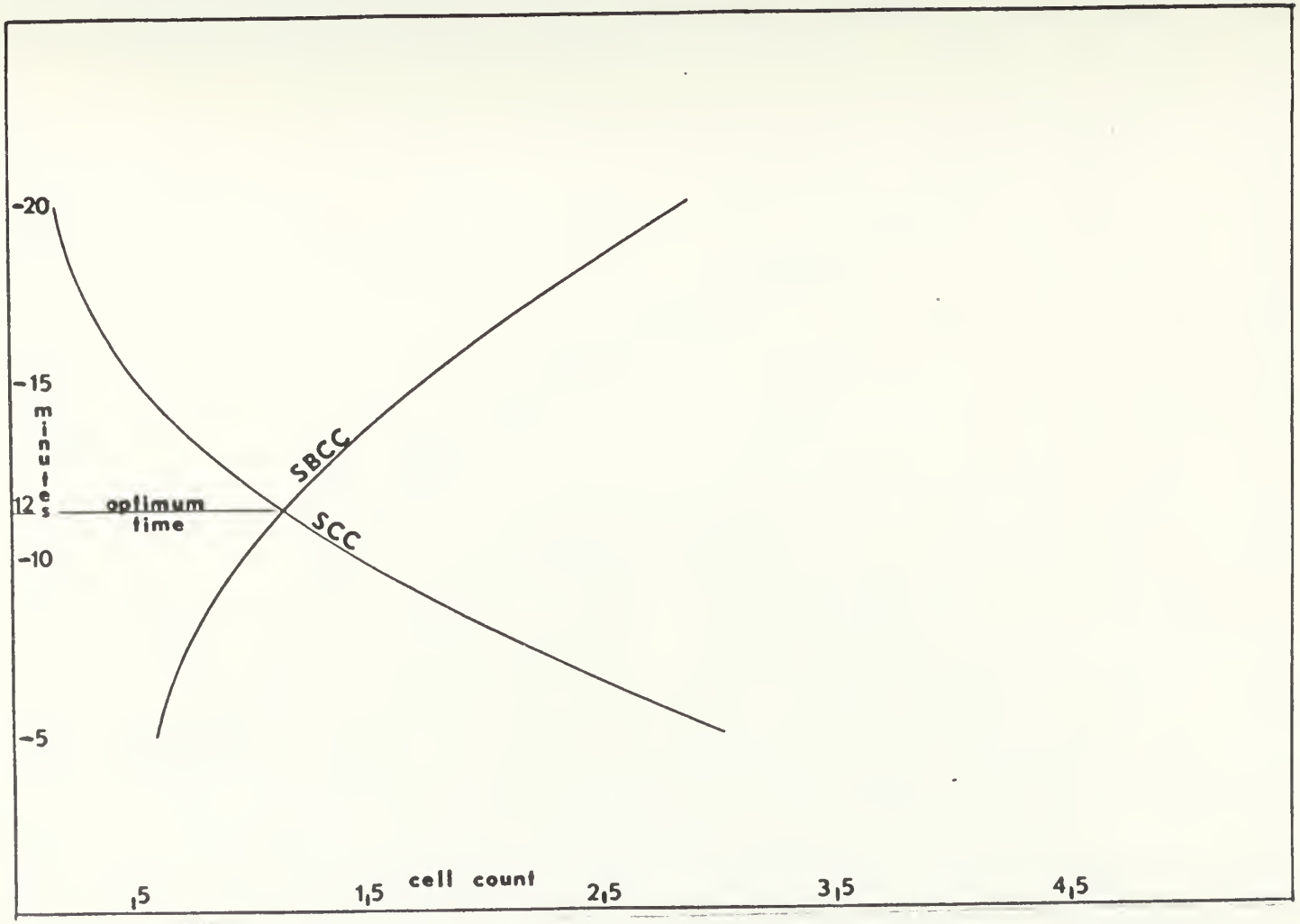


Fig. 16. Optimum centrifugation time for 1200 RPM: suspended cell count (SCC) and sediment broken cell count (SBCC) versus length of centrifugation (min).

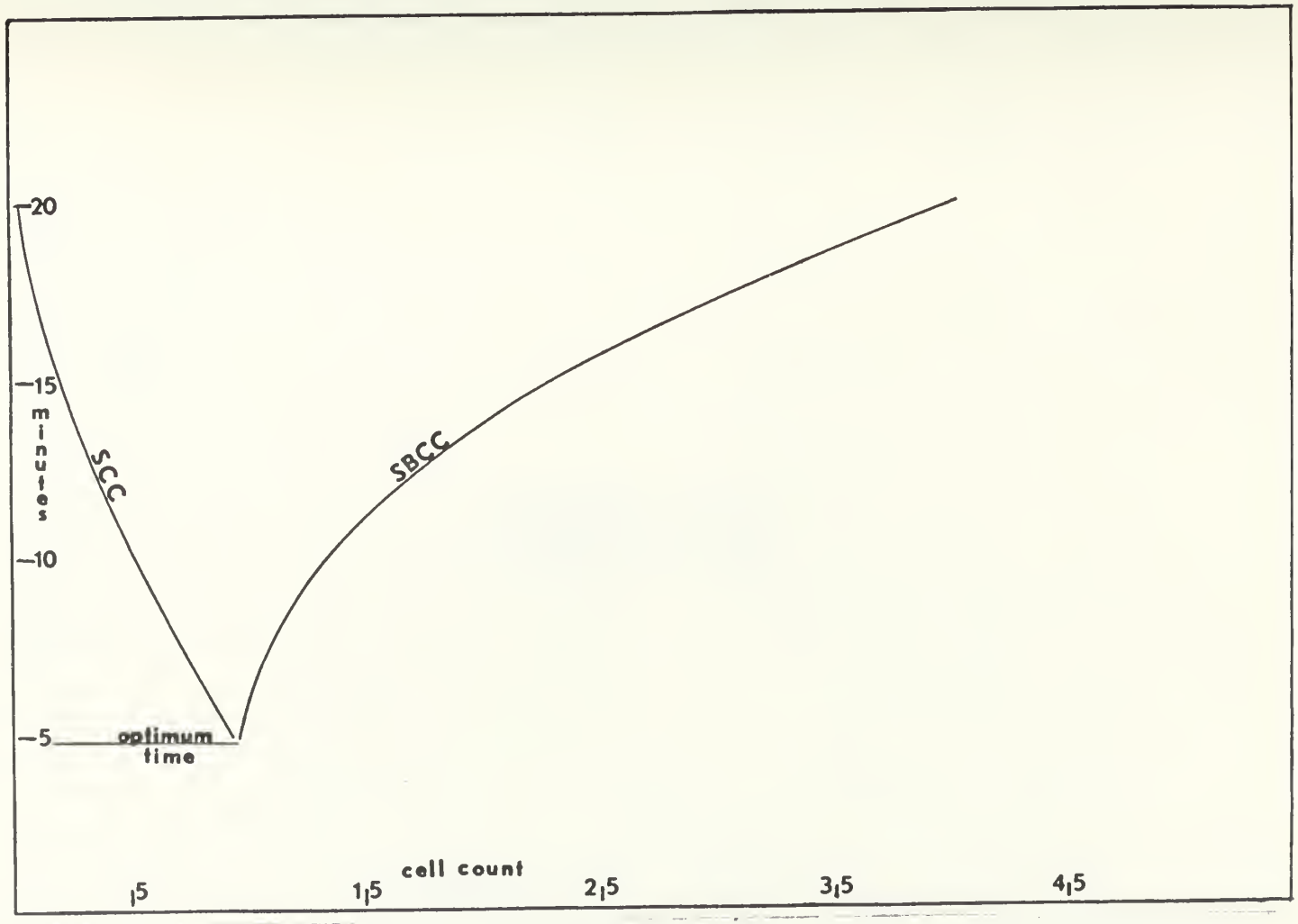


Fig. 17. Optimum centrifugation time for 1800 RPM: suspended cell count (SCC) and sediment broken cell count (SBCC) versus length of centrifugation (min).

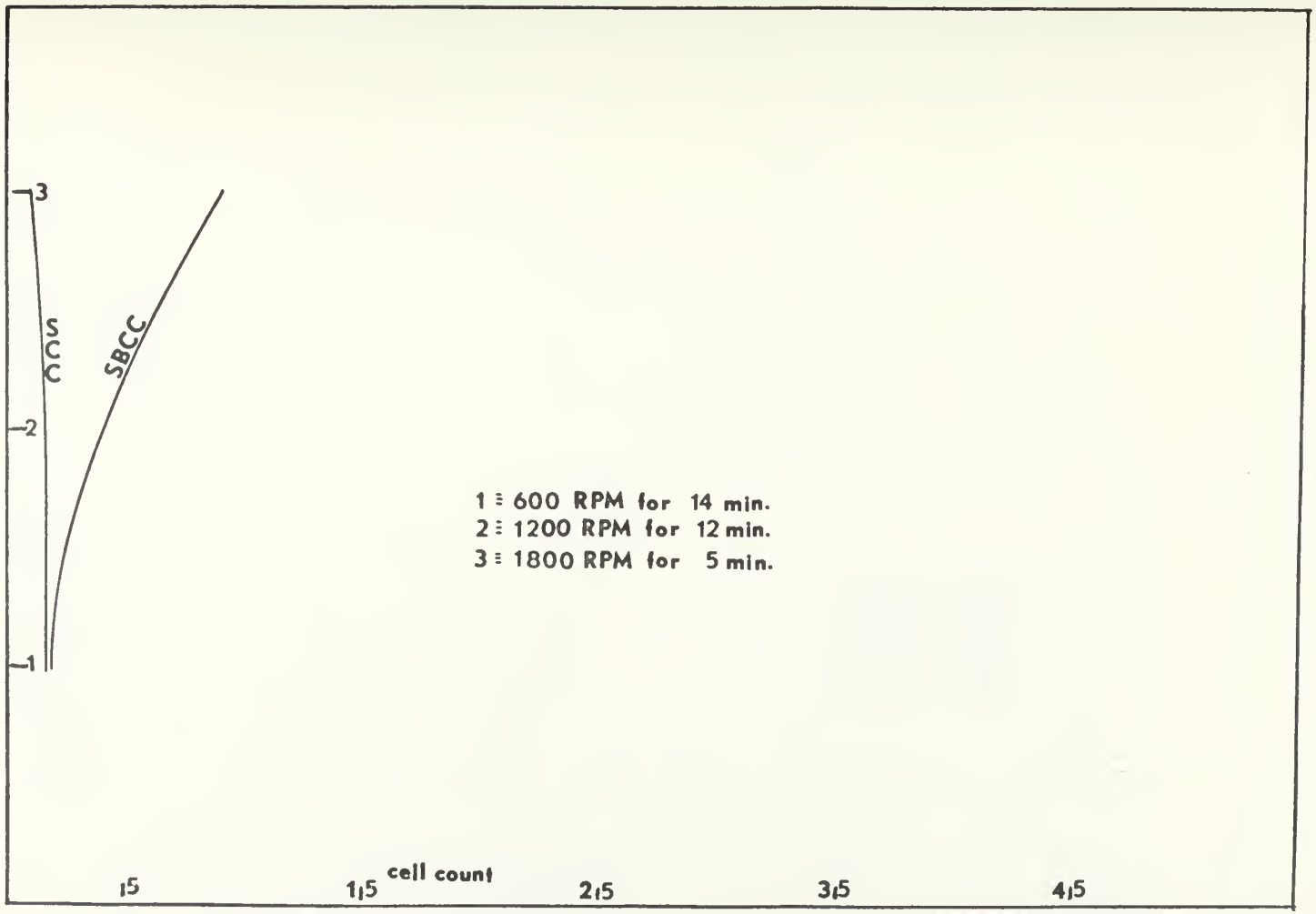


Fig. 18. Optimum centrifugation time and rate: suspended cell count (SCC) and sediment broken cell count (SBCC) versus optimum centrifugation time for 600 RPM, 1200 RPM, and 1800 RPM.

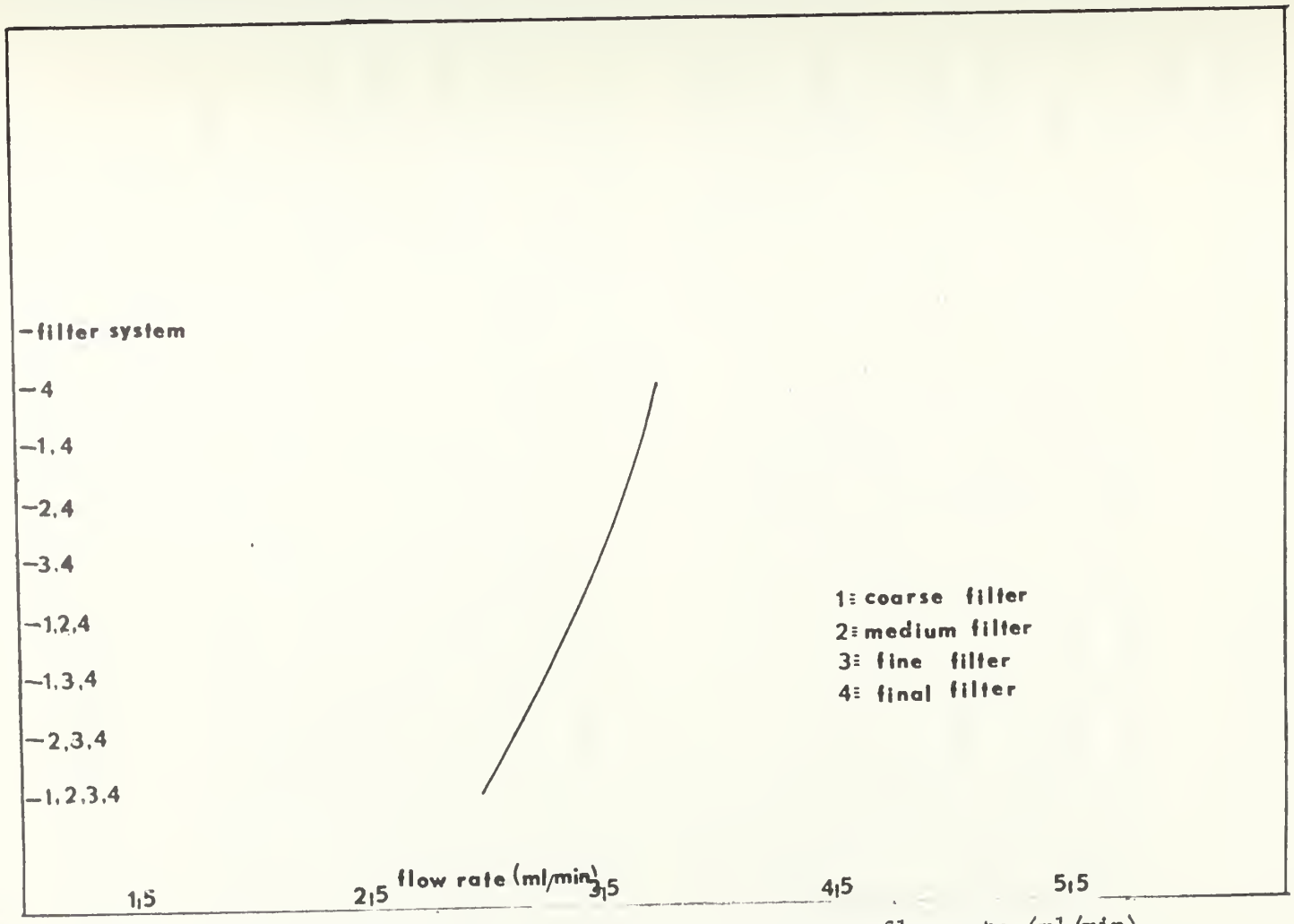


Fig. 19. Filtration-centrifugation filter system versus flow rate (ml/min).

distinct spots developed after phenolic (Fig. 20) and amino (Fig. 20) controls were processed. These patterns were compared with the sample chromatograms. The position and color of these spots permitted their recognition from the spots produced by dissolved organics in the water samples.

Some variation, e.g. "tailing," occurred in replicate chromatograms of some stations, and representative chromatograms were selected when this happened. The chromatographic results for each station were reproduced in Figs. 21 and 22. Fig. 44 is a composite of the characteristic spots for the functional groups from the individual station chromatograms consolidated into chemically similar areas when tests were repeated. The chromatographic results for each station area tabulated in Table IV.

C. ULTRA-VIOLET SPECTRA

The UV-spectra of the sea water samples (Fig. 25-34) were analysed in order to determine the wavelengths of maximum absorbance. The different wavelengths were recorded in Table V, with the corresponding sample exhibiting this absorbance and a list of the possible chemical groups responsible for such an absorbance as described by Dyer (1965). The UV-spectra of the organic standards, L-tyrosine, L-tryptophan and p-hydroxybenzoic acid are shown in Figures 35, 36, and 37.

D. DISSOLVED ORGANIC CONTENT

The calculated dissolved organic concentrations for each sea water station is listed in Table IV.

E. ORGANIC STANDARDS

The results from the experiments on the standards, paper chromatography, UV-spectrometery, and Beer's Law percent recovery, are tabulated in Table II.

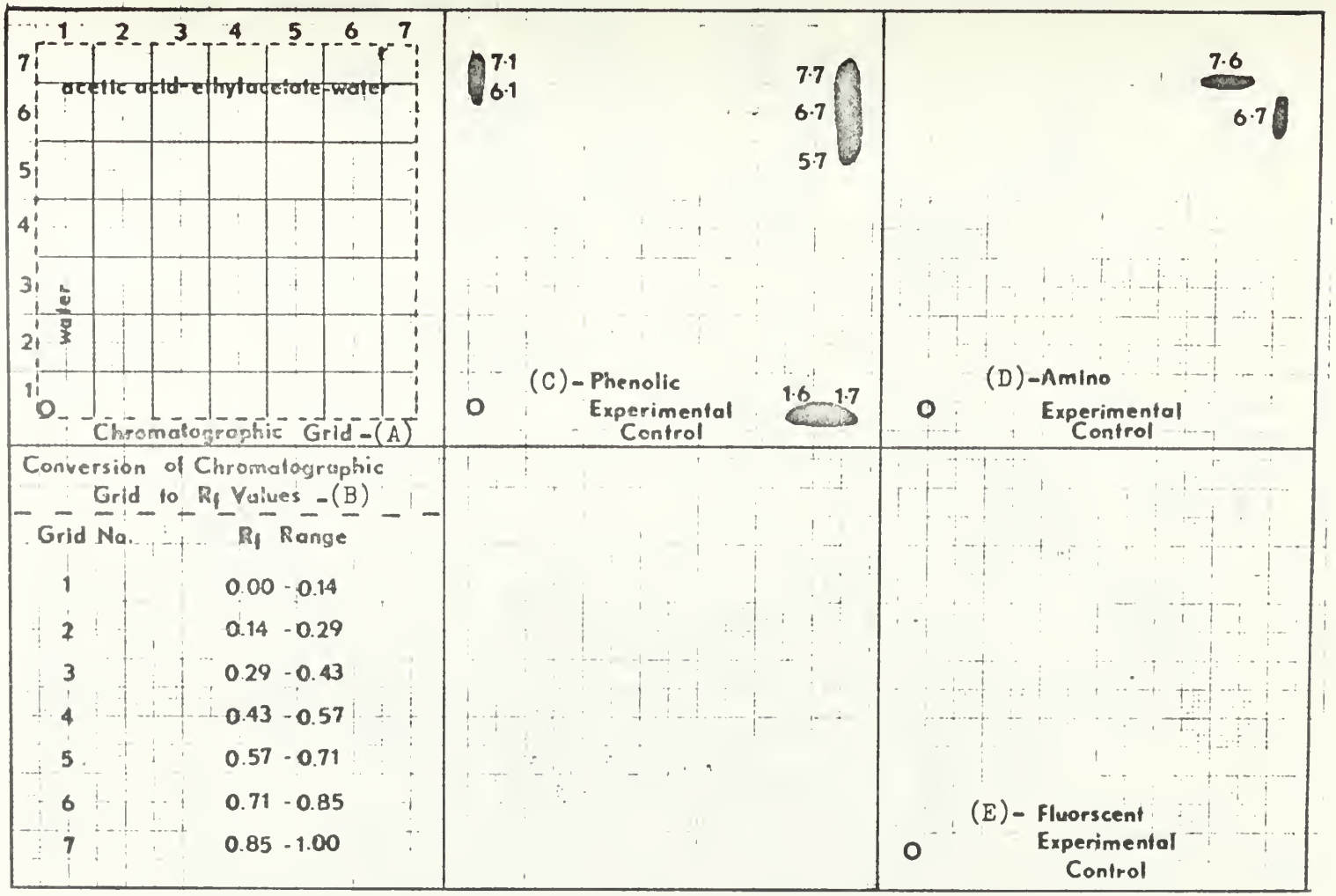


Fig.20. Chromatographic grid (A) for two dimensional paper chromatography and corresponding R_f values (B). Two dimensional paper chromatograms of phenolic experimental control (C), amino experimental control (D), and fluorescent experimental control (E).

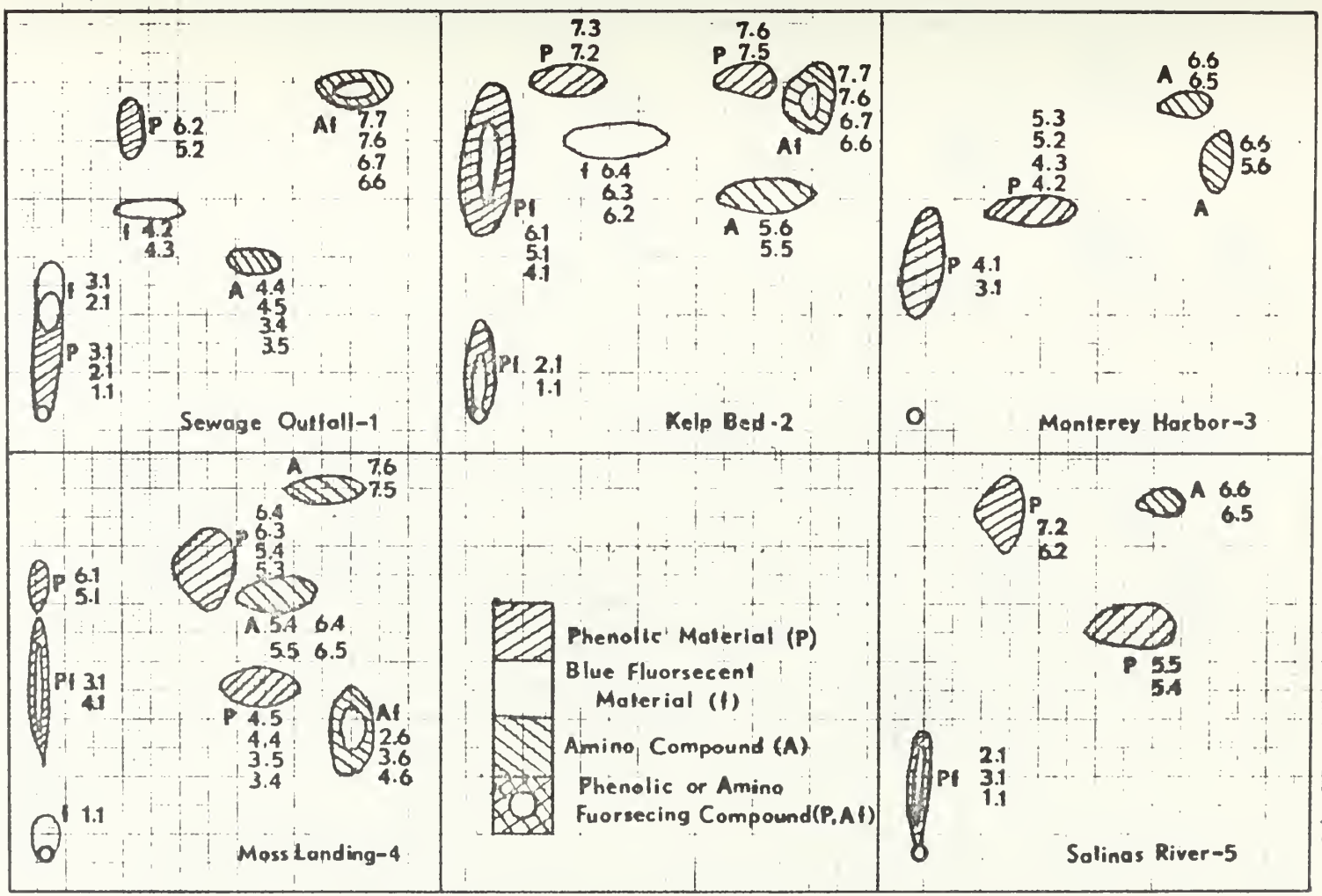


Fig.21. Representative two dimensional paper chromatograms to show patterns of phenolic, amino, and fluorescent compounds in nylon column eluates of sea water from sampling stations.

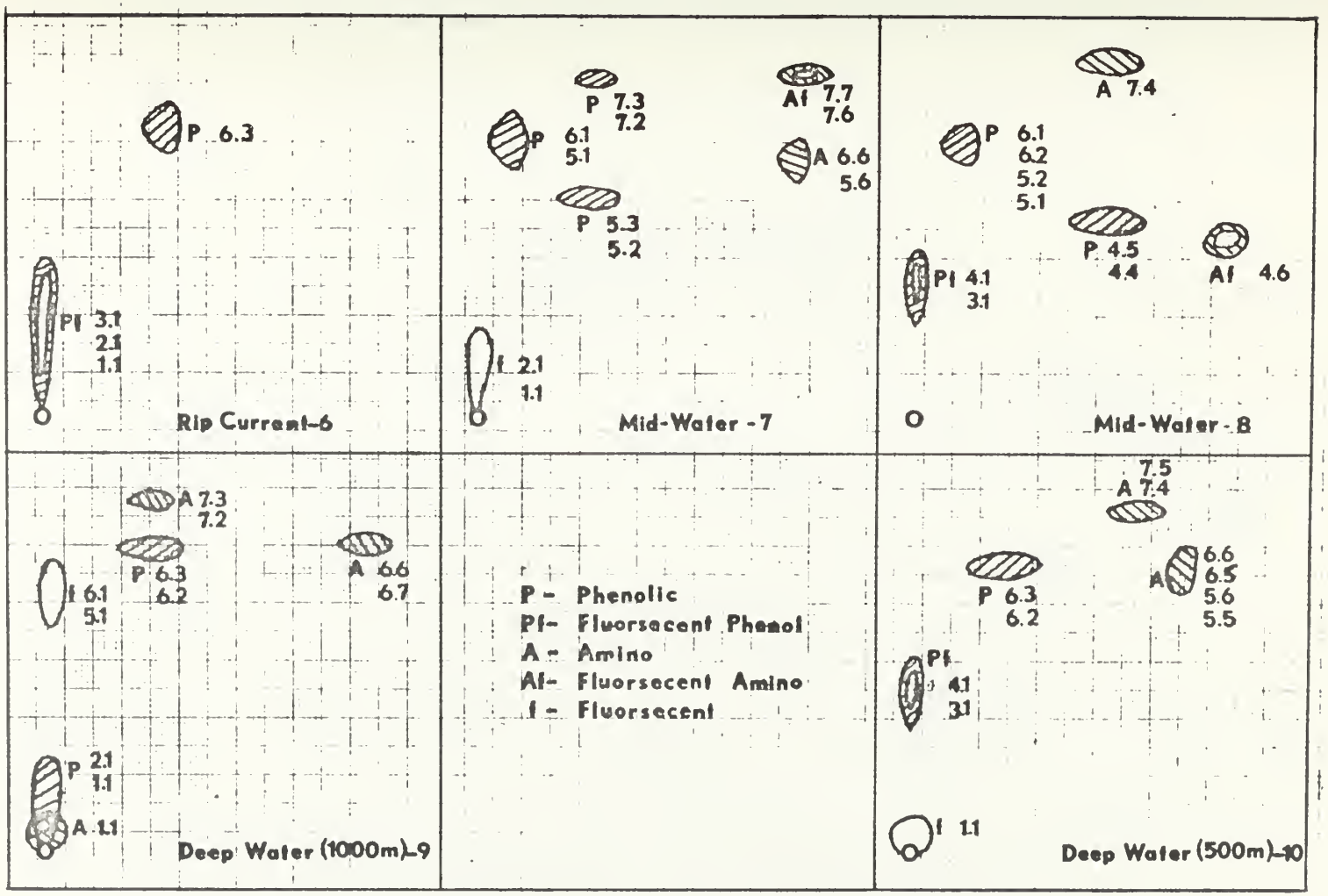


Fig.22. Representative two dimensional paper chromatograms to show patterns of phenolic, amino, and fluorescent compounds in nylon column eluates of sea water from sampling stations.

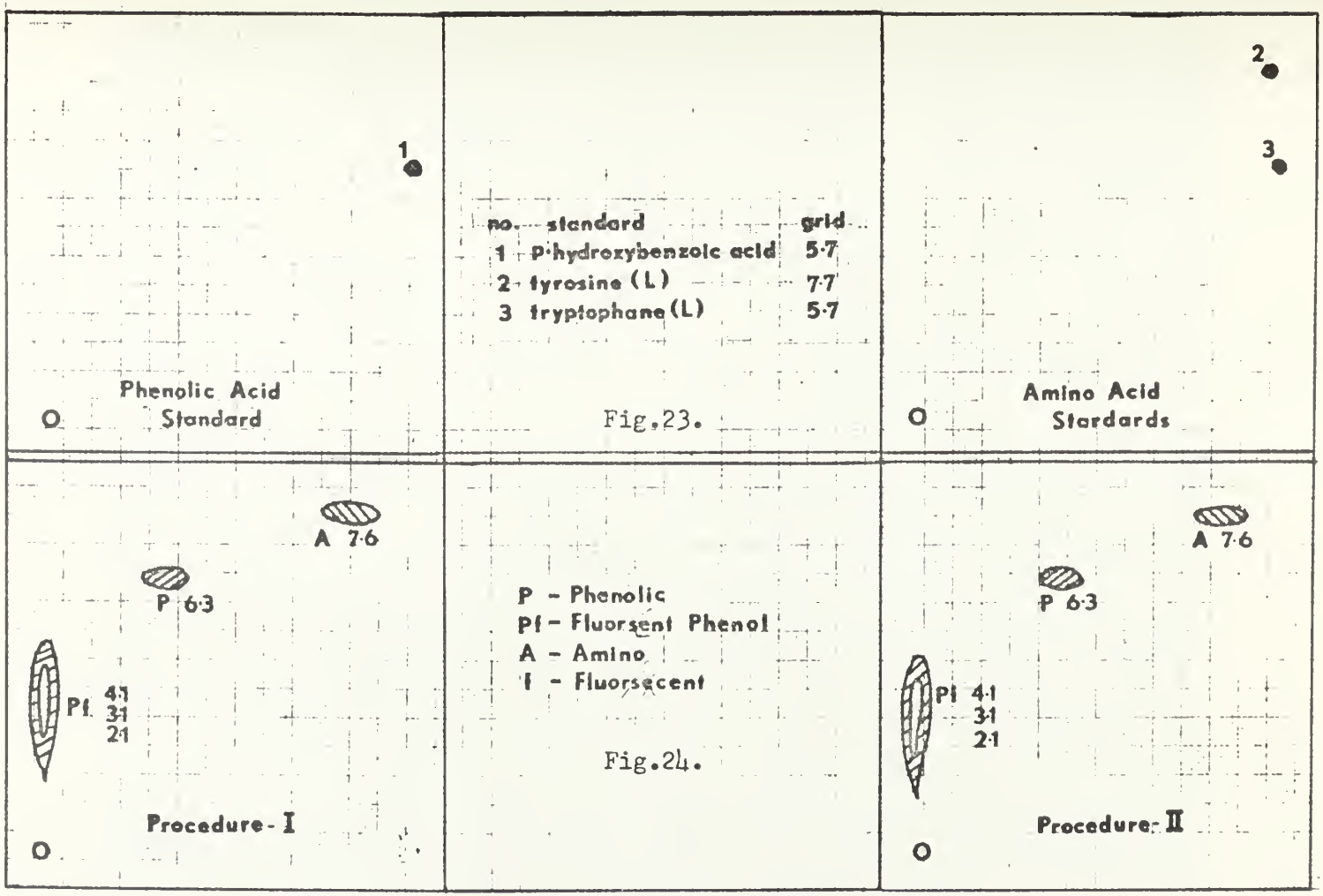


Fig.23. Two dimensional paper chromatograms of organic standards.
Fig.24. Two dimensional paper chromatograms to show patterns of phenolic, amino, and fluorescent compounds from Filtration Procedure I and Filtration-Centrifugation Procedure II.

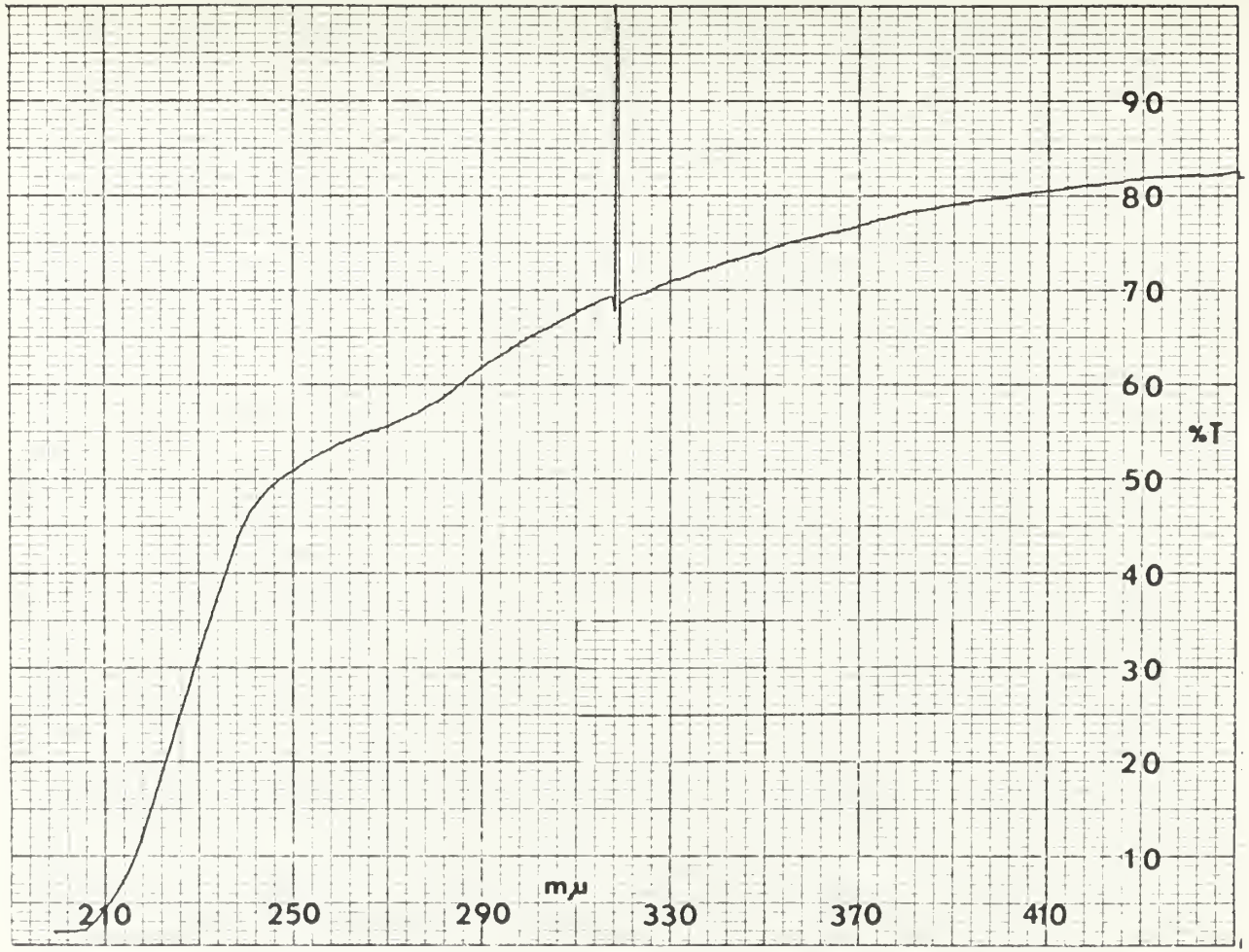


Fig.25. UV-spectrum of dissolved organic material from sewage outfall, station 1.

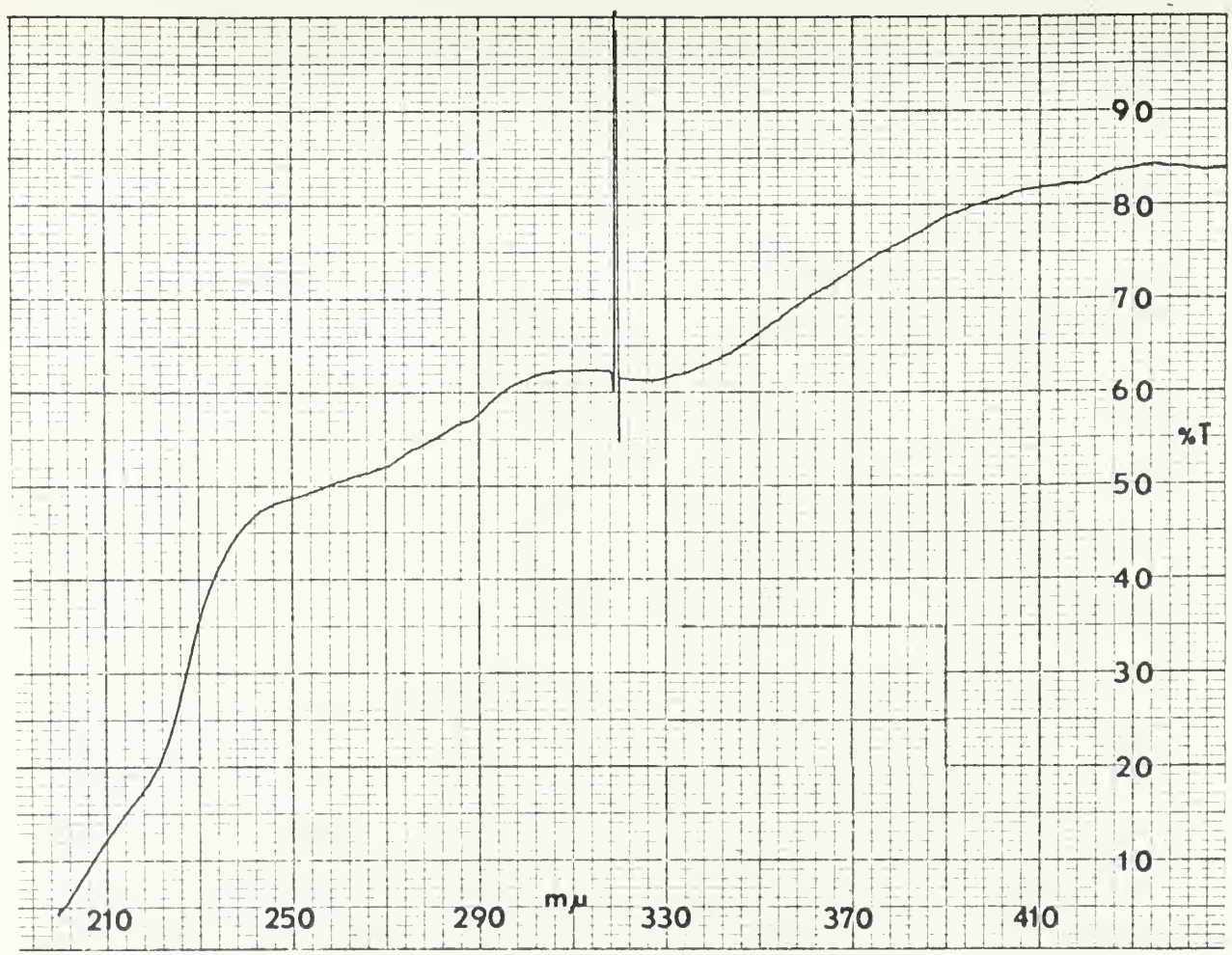


Fig.26. UV-spectrum of dissolved organic material from kelp beds, station 2.

Fig.27. UV-spectrum of dissolved organic material from Monterey Harbor, station 3.

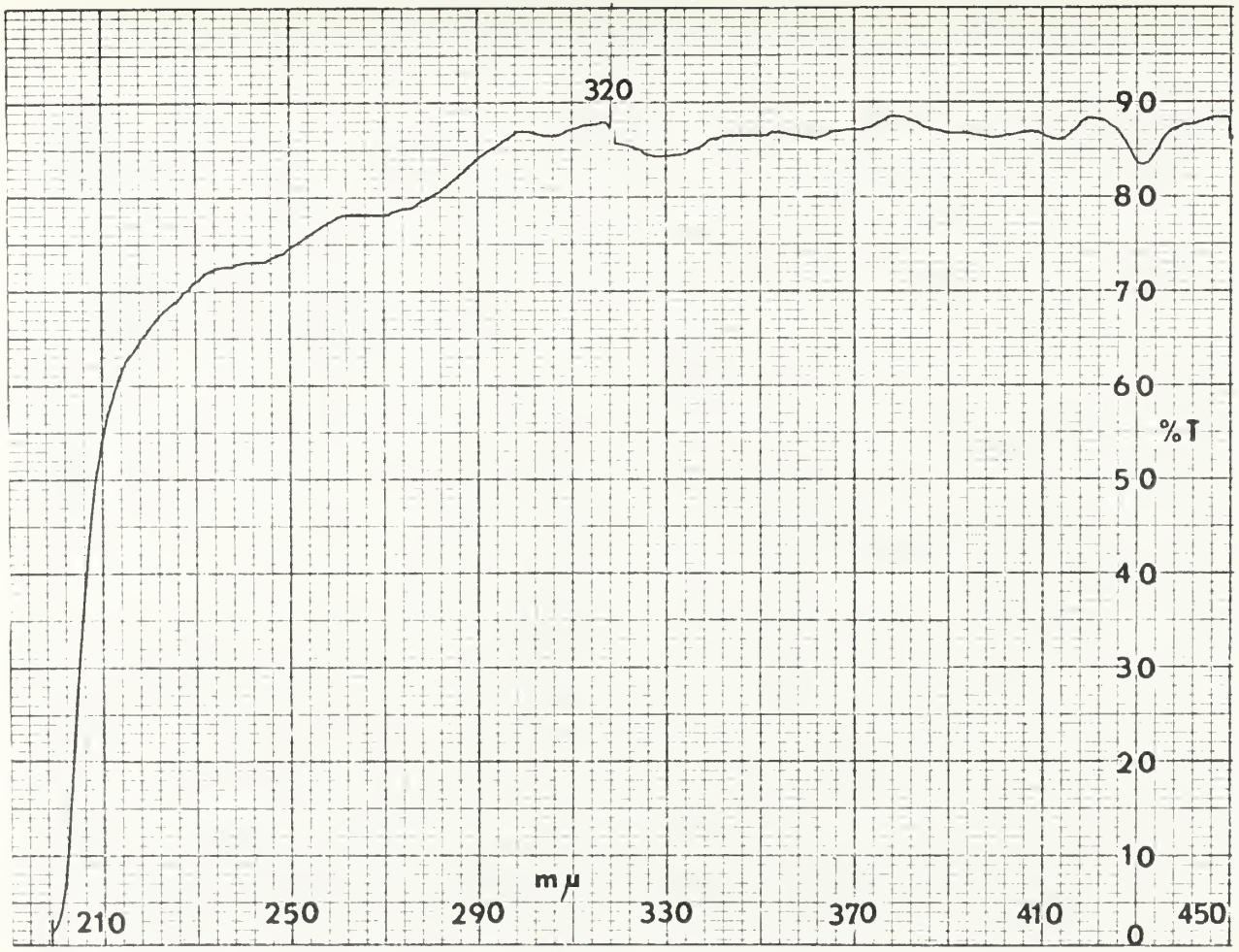


Fig.28. UV-spectrum of dissolved organic material from Moss Landing, station 4.

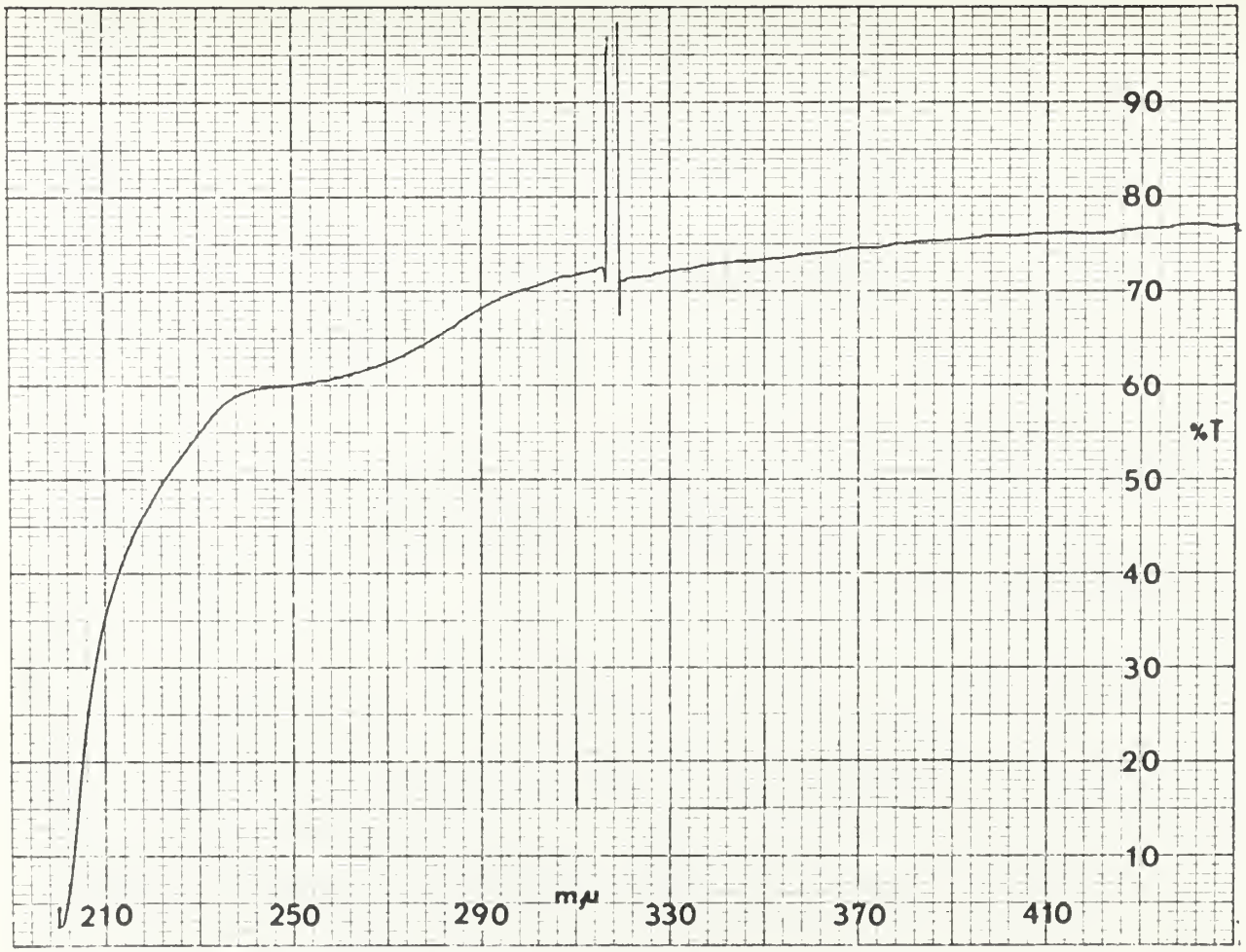


Fig.29. UV-spectrum of dissolved organic material from Salinas River, station 5.

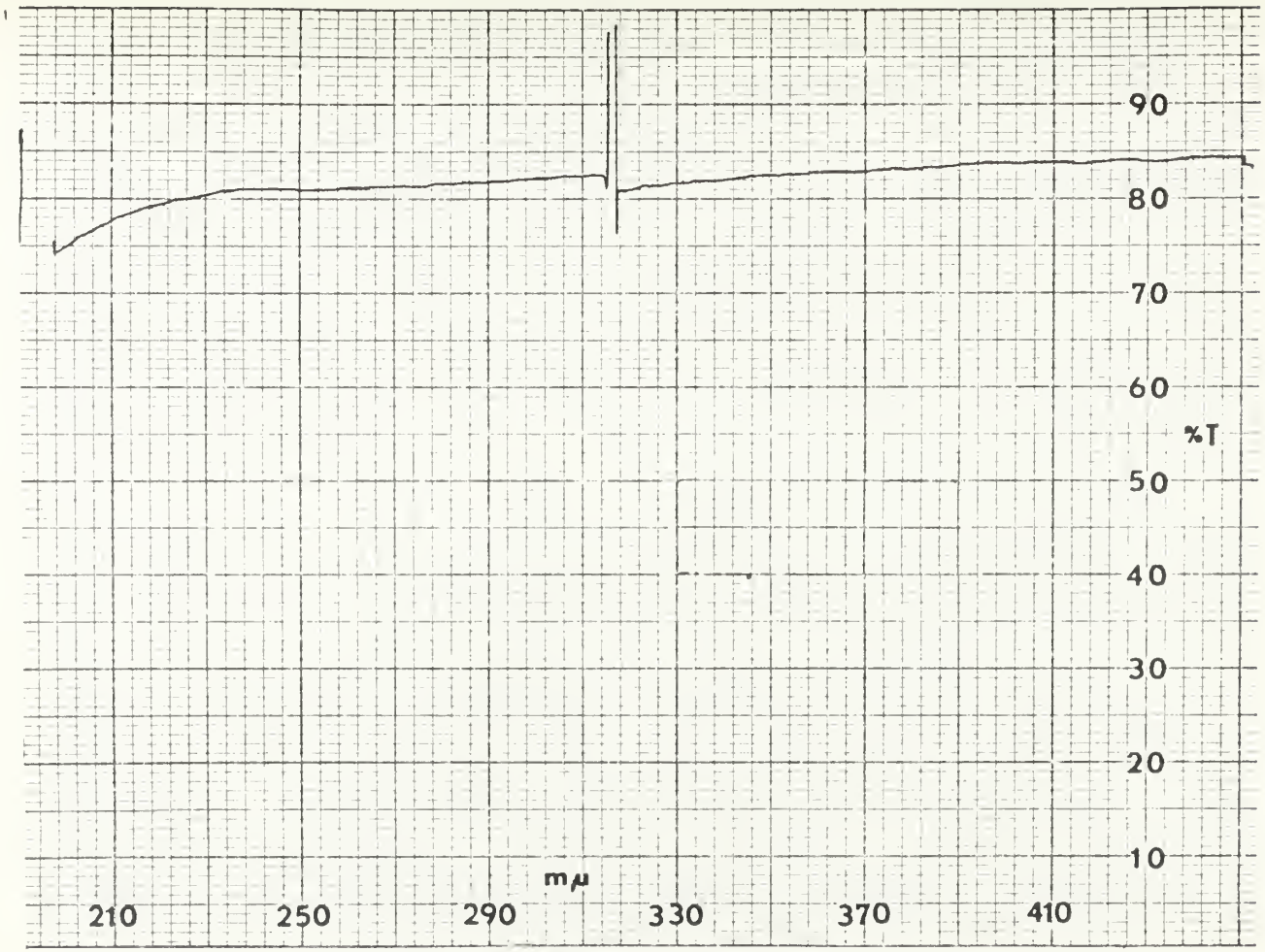


Fig.30. UV-spectrum of dissolved organic material from rip current, station 6.

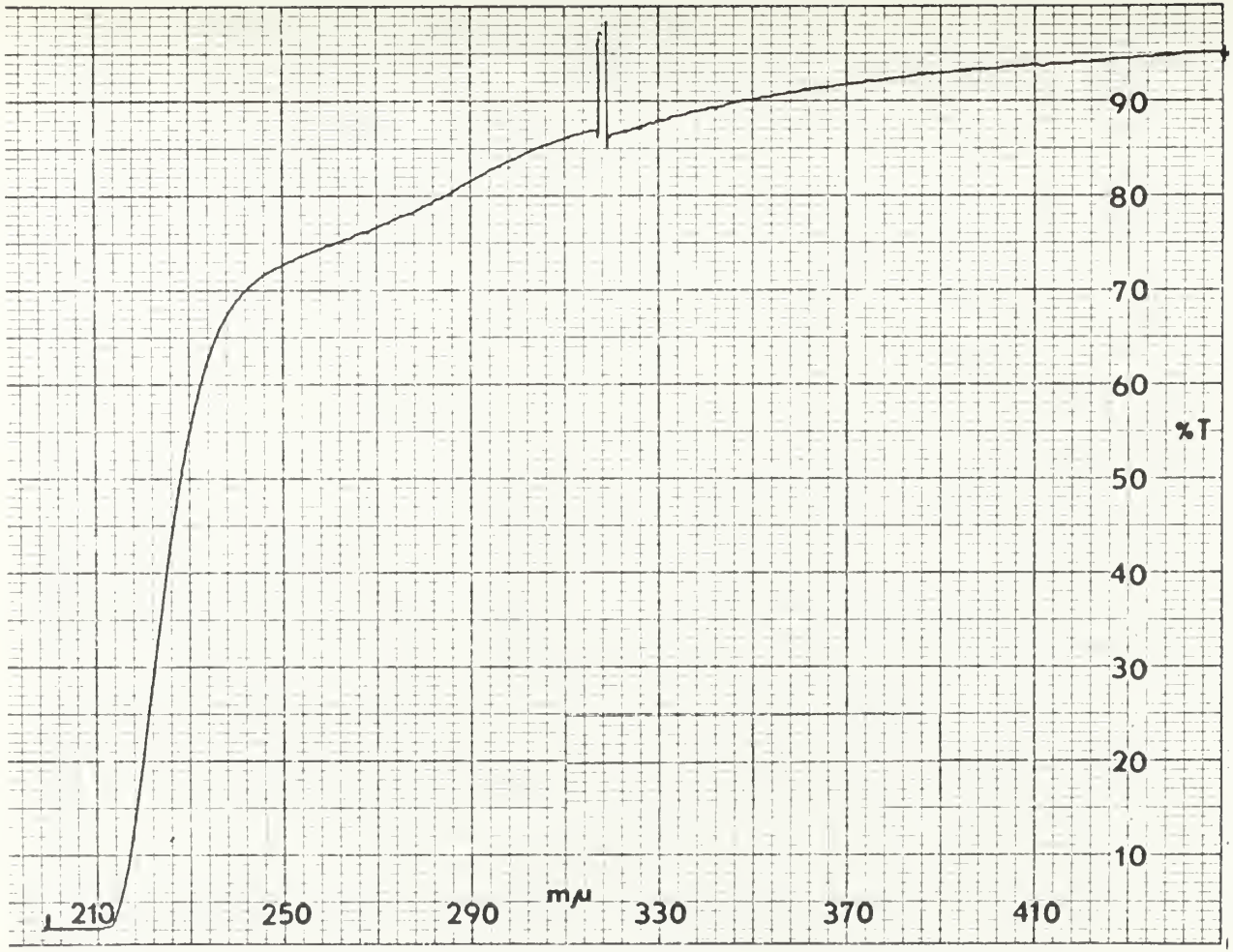


Fig.31. UV-spectrum of dissolved organic material from mid-water, station 7.

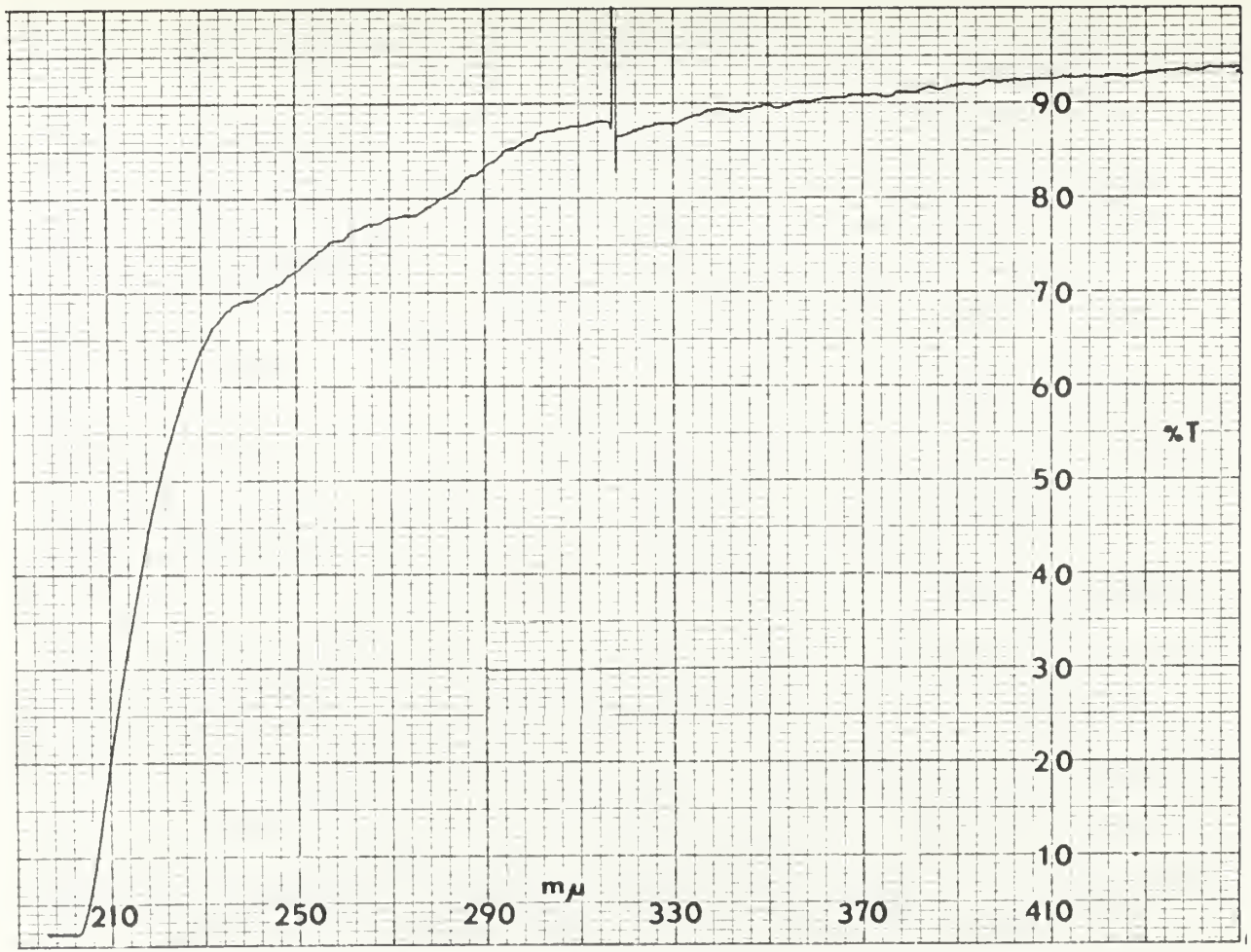


Fig.32. UV-spectrum of dissolved organic material from mid-water, station 8.

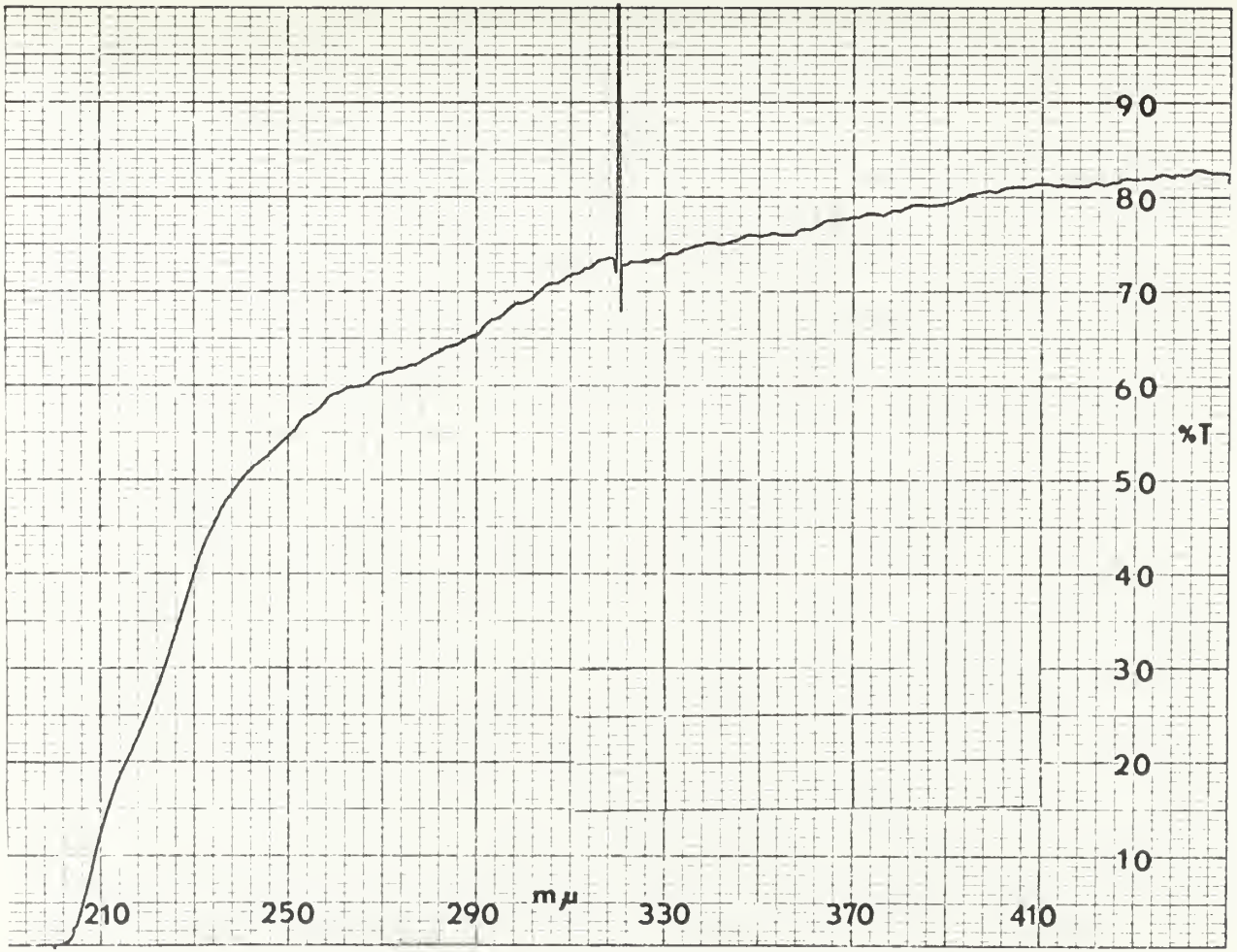


Fig.33. UV-spectrum of dissolved organic material from deep water, station 9.

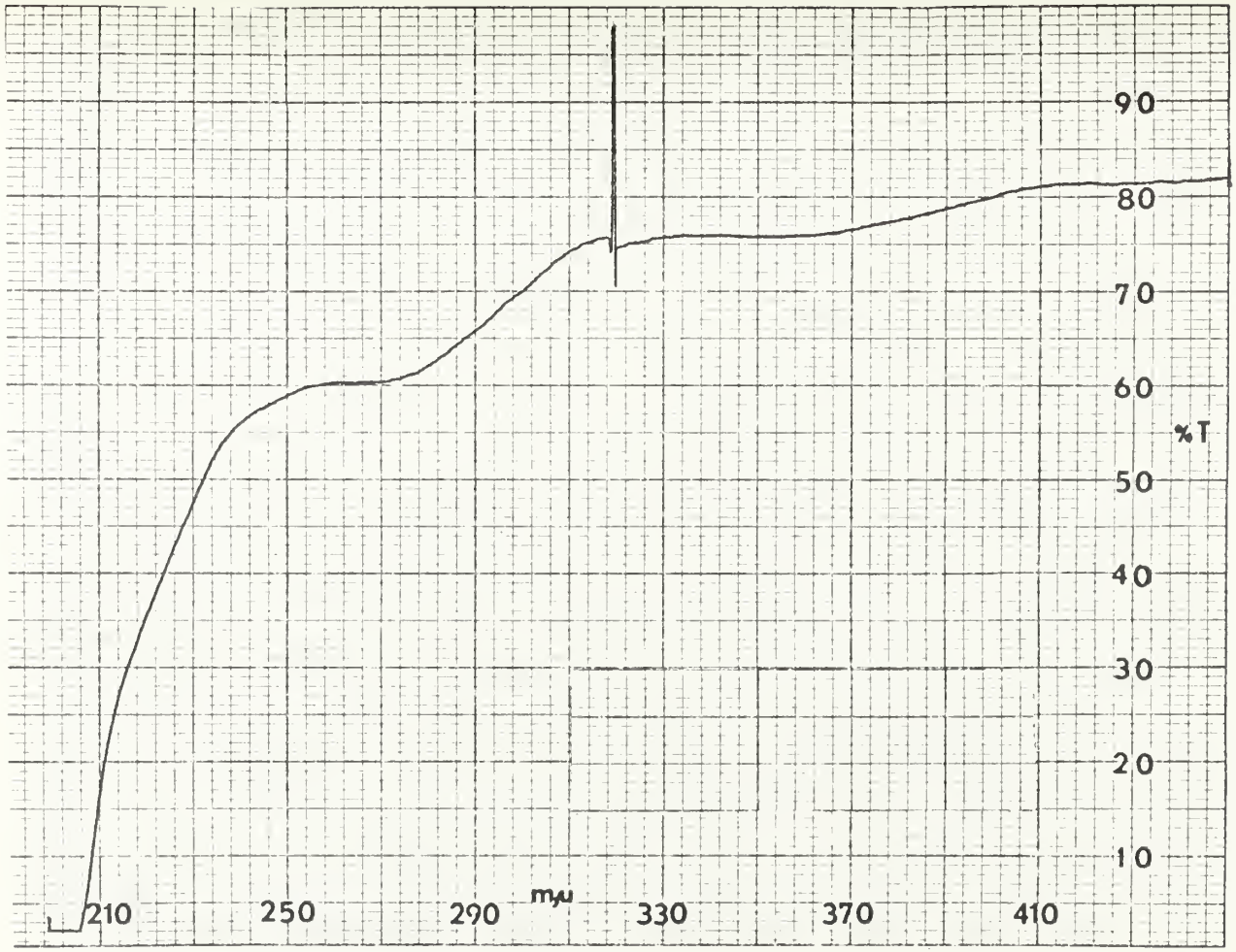


Fig.34. UV-spectrum of dissolved organic material from deep water, station 10.

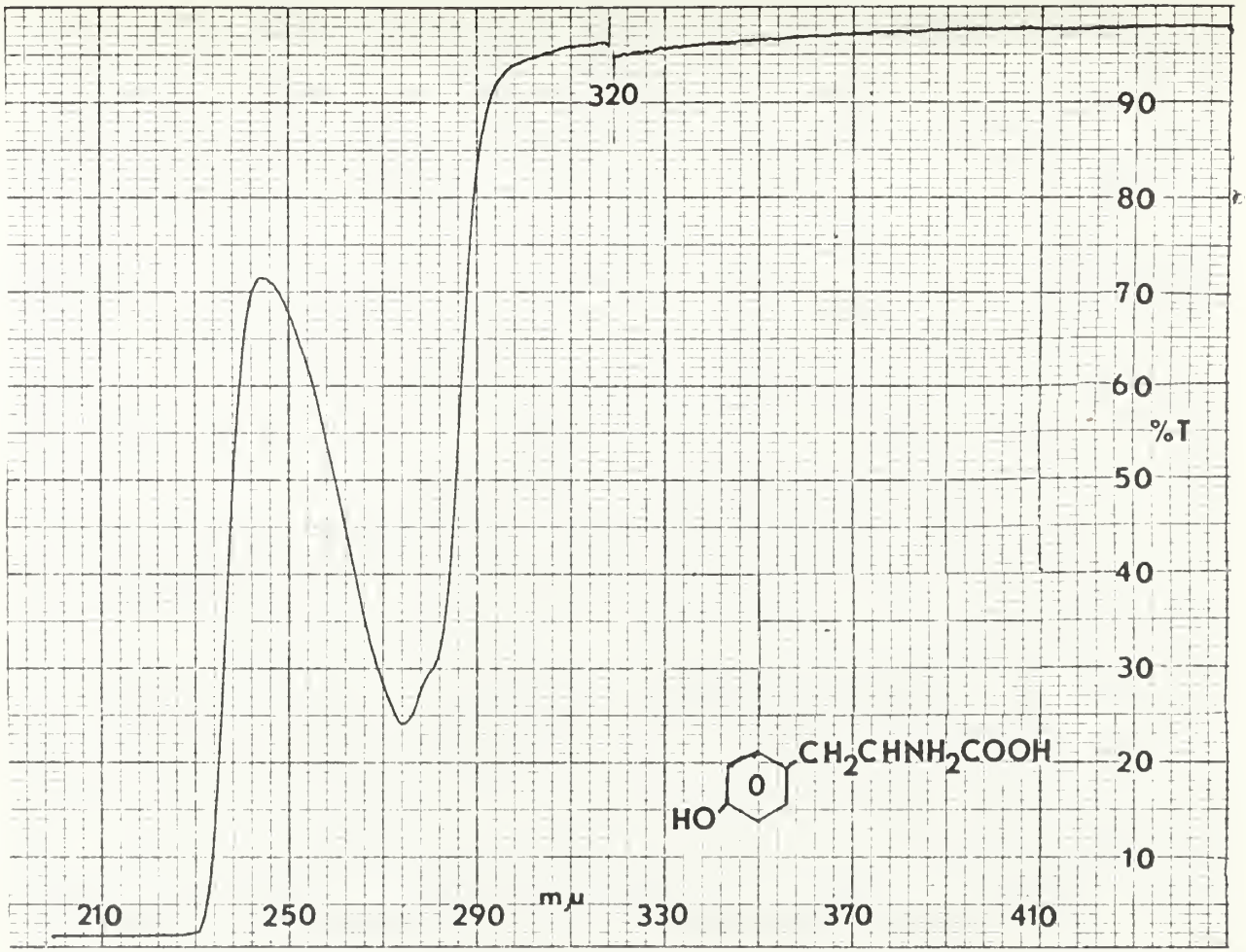


Fig.35. UV-spectrum of L-tyrosine.

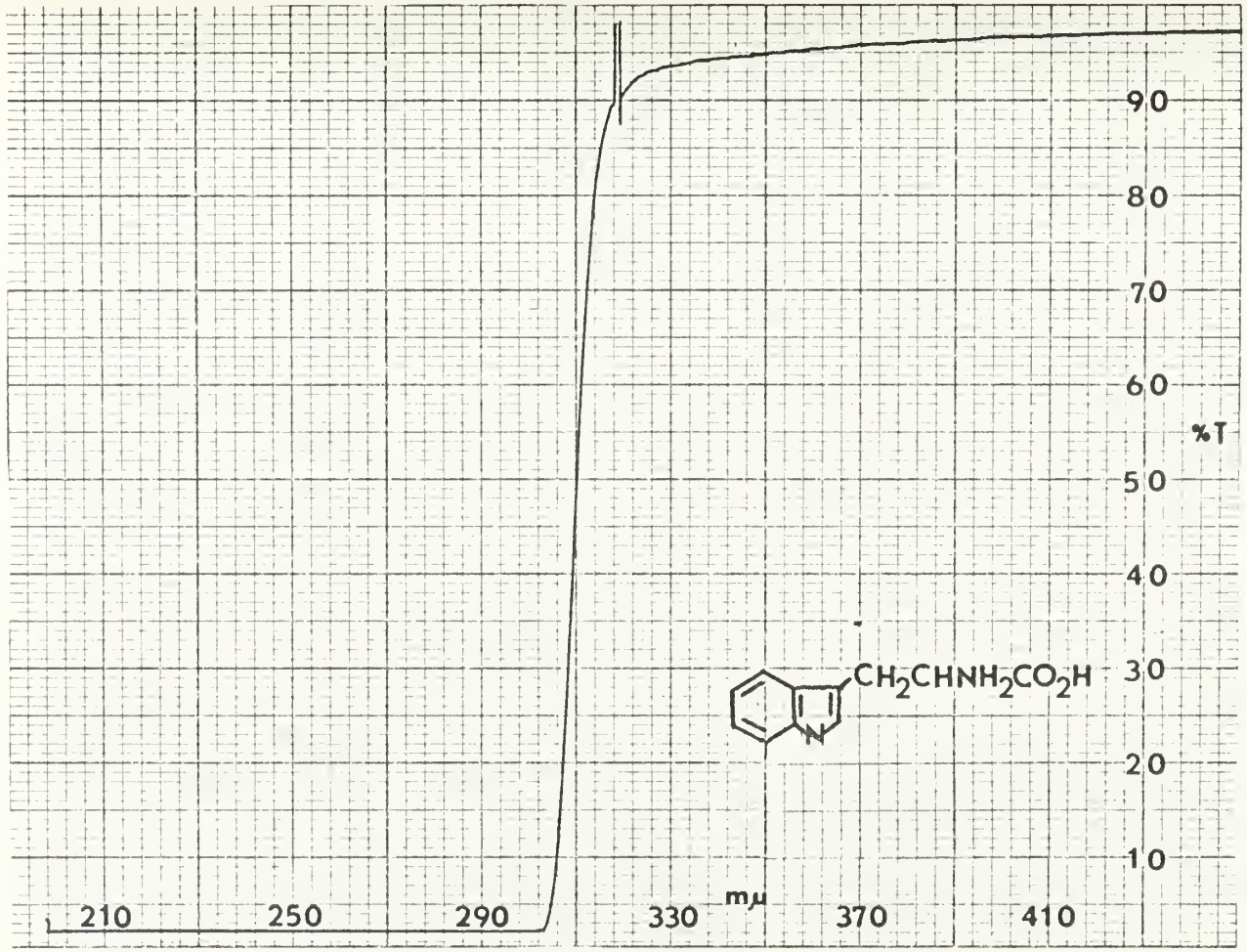


Fig.36. UV-spectrum of L-tryptophan.

Fig.37. UV-spectrum of p-hydroxybenzoic acid.

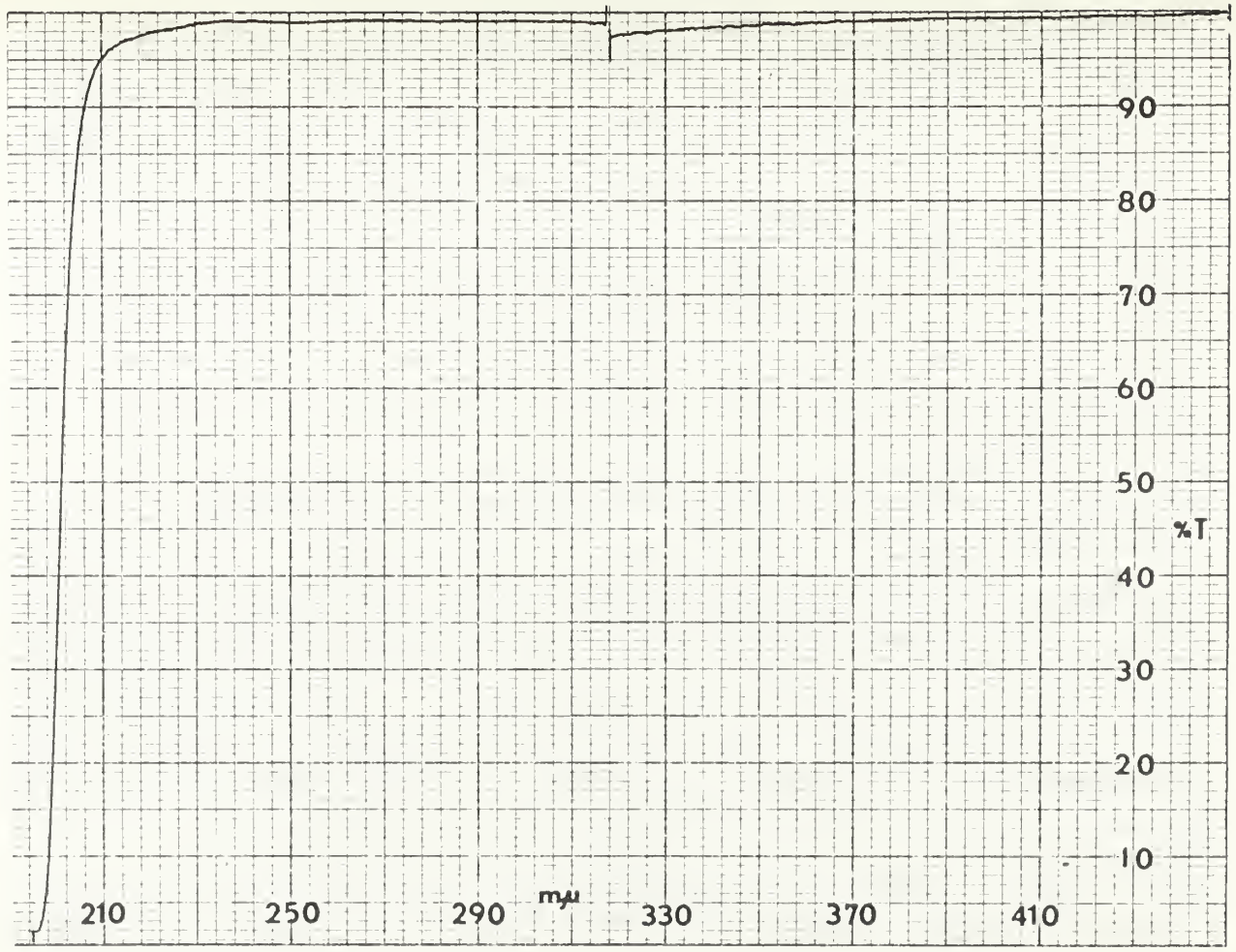


Fig.38. UV-spectrum of dissolved organic material from the experimental control, e.g. distilled water passed through the column acidified and them neutralized.

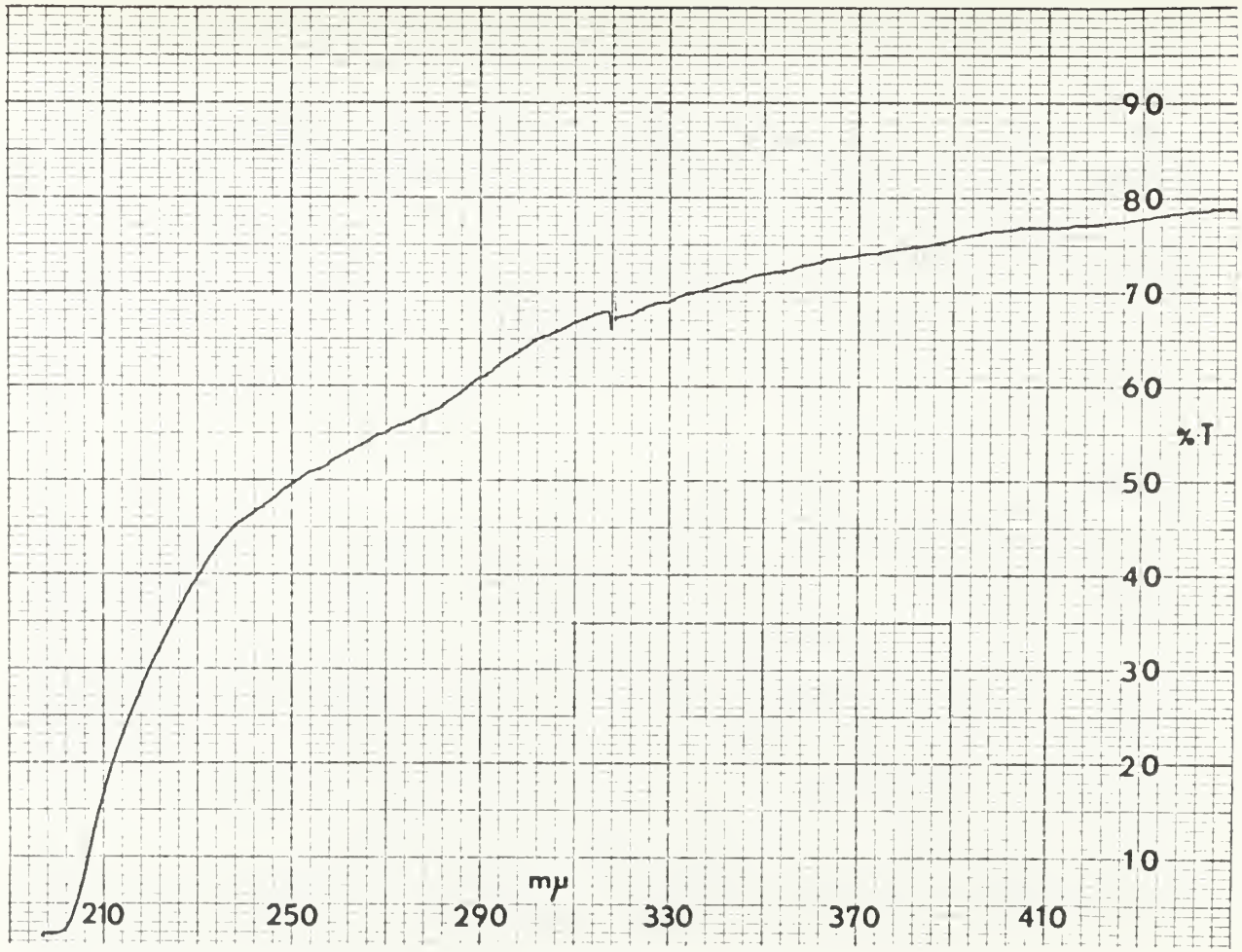


Fig.39. UV-spectrum of dissolved organic material from the filtration of raw sea water.

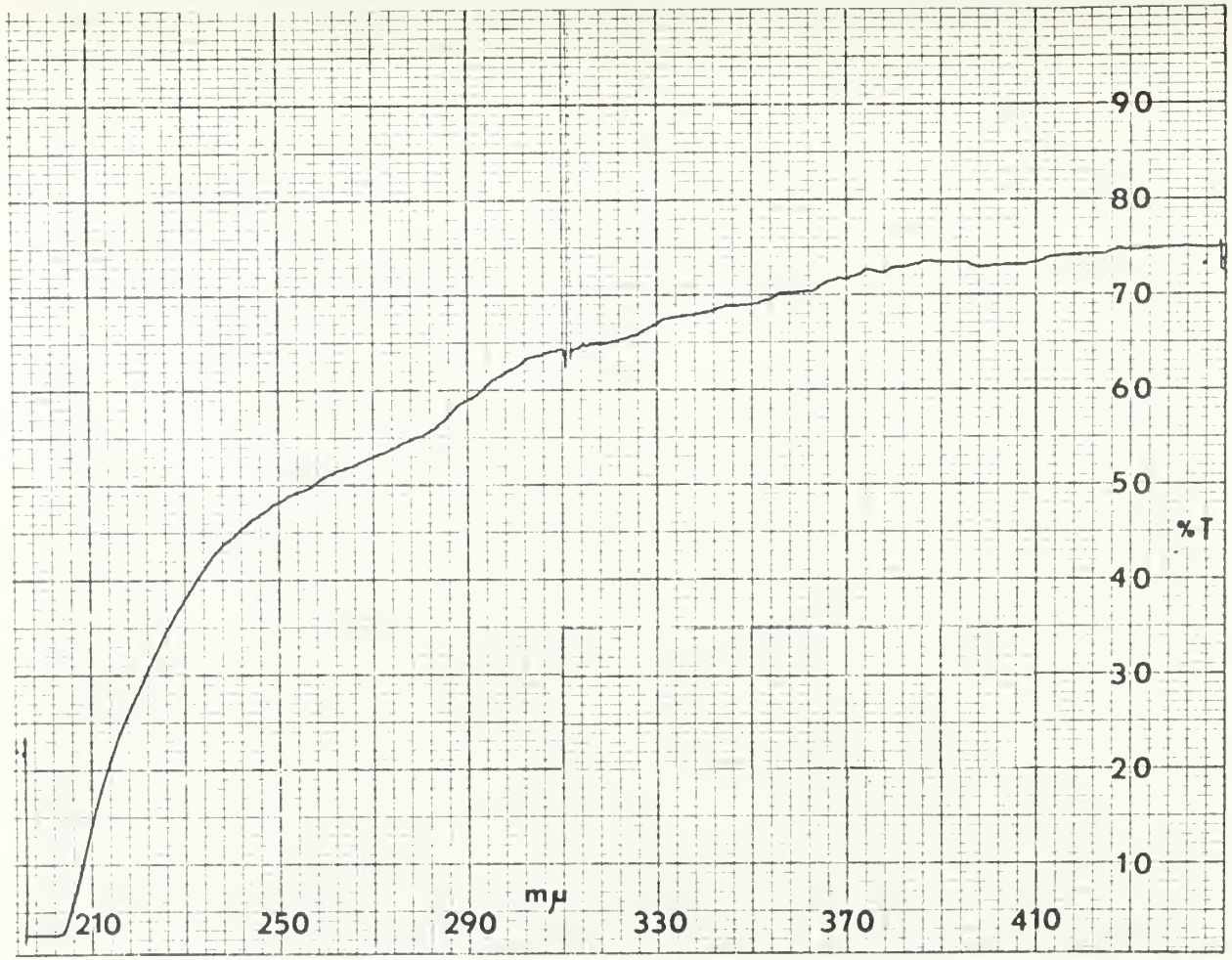


Fig.40. UV-spectrum of dissolved organic material from the of raw sea water.

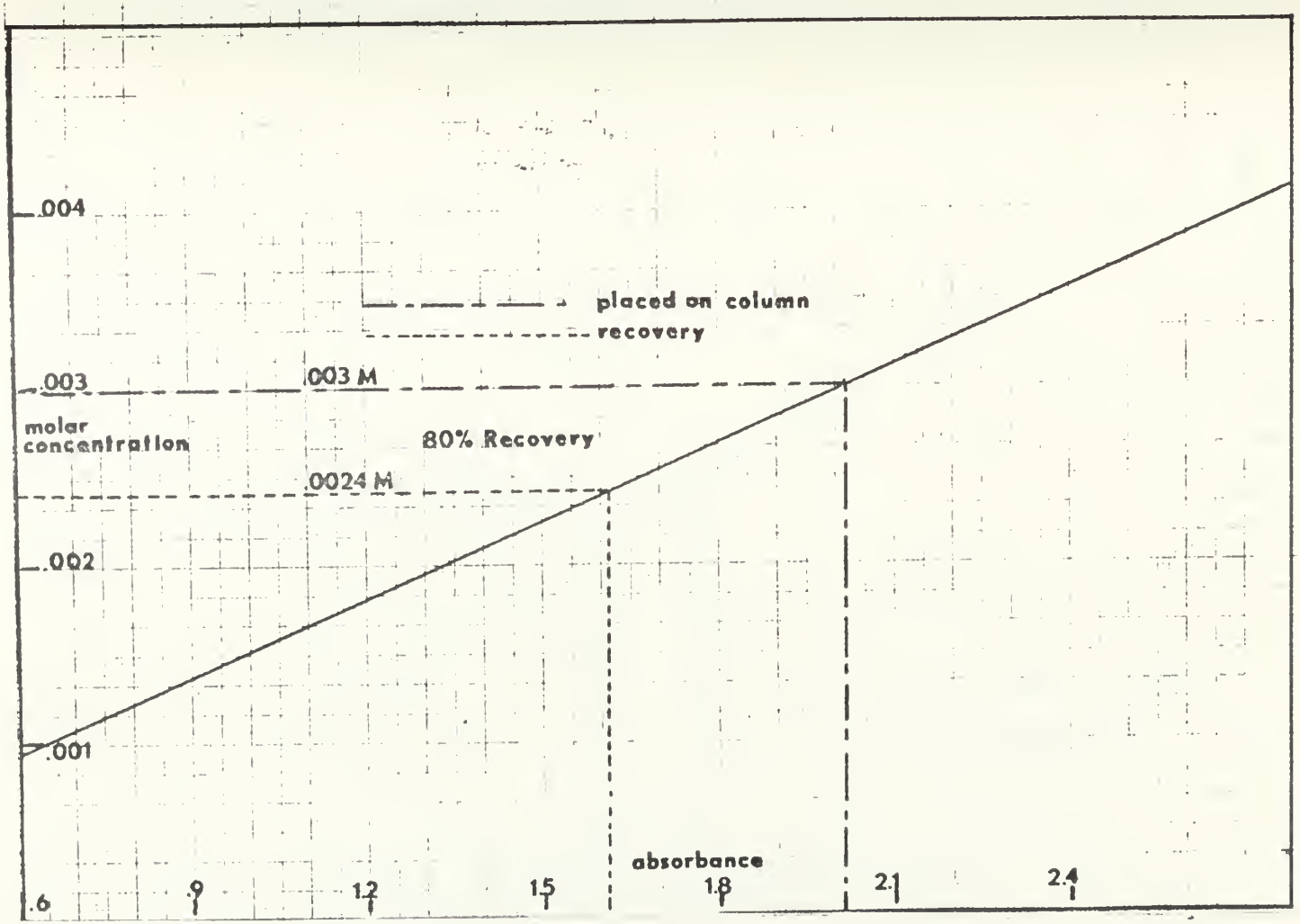


Fig. 41. Nylon column recovery of L-tyrosine based on a Beer's law graph of molar concentration versus absorption at the wavelength of 274 millimicrons mechanically plotted for four known concentrations.

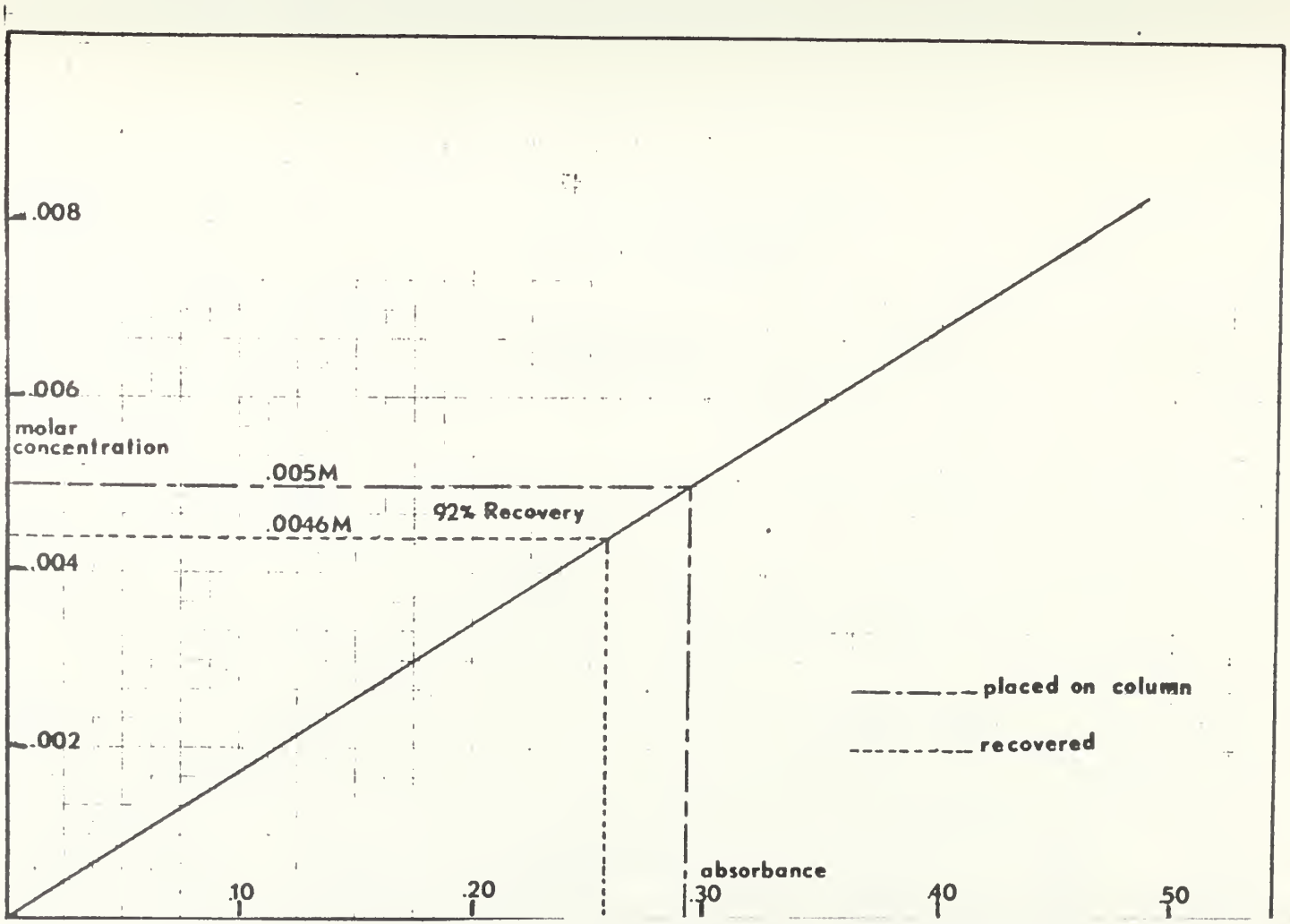


Fig. 42. Nylon column recovery of L-tryptophan based on a Beer's law graph of molar concentration versus absorption at the wavelength of 540 millimicrons mechanically plotted for four known concentrations.

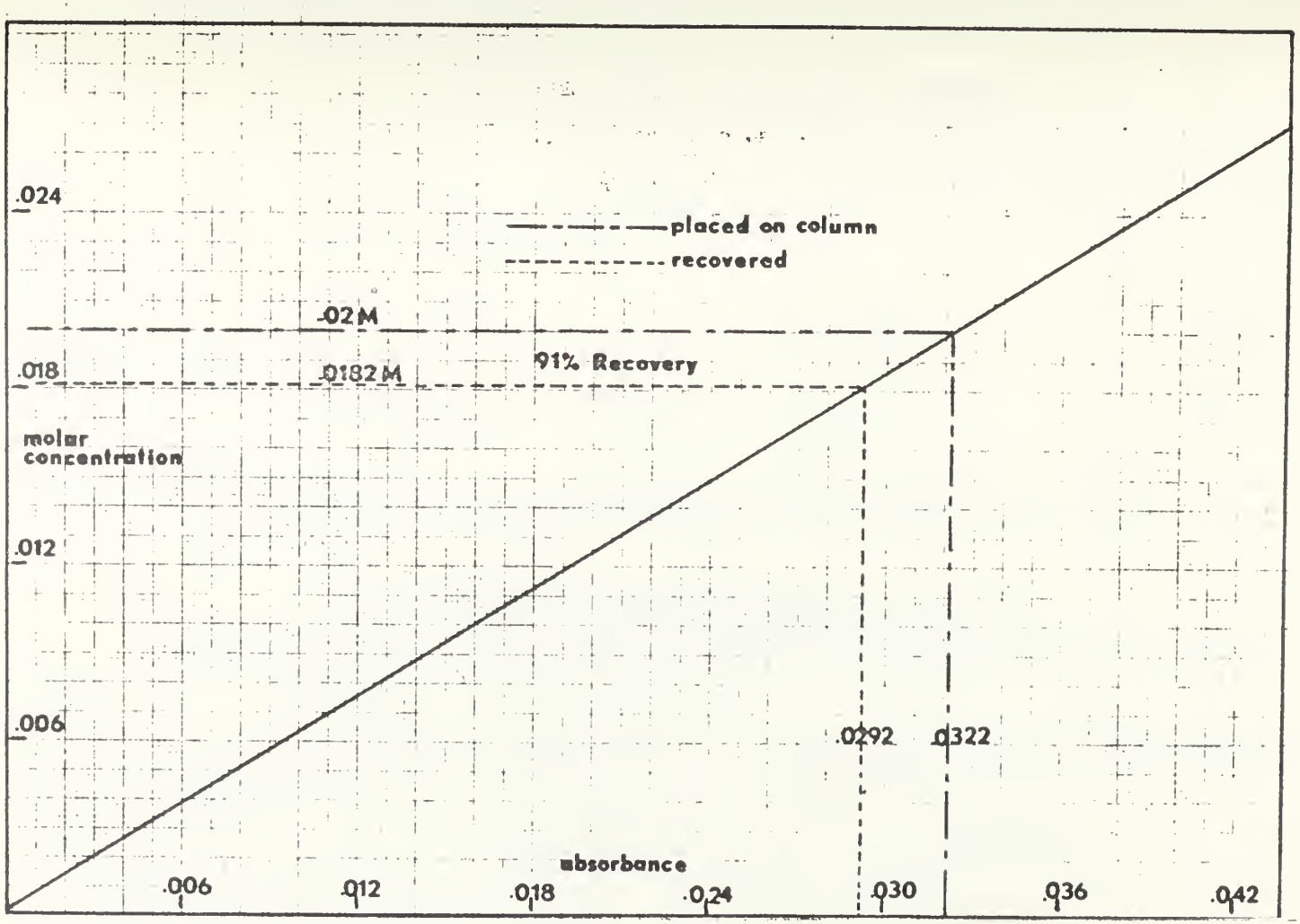


Fig. 43. Nylon column recovery of p-hydroxybenzoic acid based on a Beer's law graph of molar concentration versus absorption at the wavelength of 780 millimicrons mechanically plotted for four known concentrations.

Table II: EXPERIMENTAL RESULTS FOR ORGANIC STANDARDS

Standard	R_f	Grid No.	UV-Absorbance (mμ)	% Recovery
p-Hydroxybenzoic Acid (phenolic)	0.64-0.94	5-7	---	91%
L-Tyrosine (fluorescent amino)	0.88-0.89	7-7	274 280	80%
L-Tryptophan (fluorescent amino)	0.65-0.93	5-7	---	92%

Table III: EXPERIMENTAL RESULTS:
FILTRATION AND FILTRATION-CENTRIFUGATION METHODS COMPARISON

Analysis	Procedure I.	Procedure II.
Paper Chromatography (grid number)	P 6-3; A 7-6; Pf 4,3,2-1	P 6-3; A 7-6; Pf 4,3,2-1
UV-Absorbance (millimicrons)	278-284	280-284
Dissolved Organic Concentration (mg C/l)	1.56	1.47
Process Time (per liter)	33 min.	26 min.

Table IV:

EXPERIMENTAL RESULTS

SEA WATER SAMPLES

SAMPLE	CHROMATOGRAPHIC GRID NUMBER					UV-SPECTRUM	
	Phenol	Amino Acid	Fluorescent Phenol	Fluorescent Amino	Fluorescent	Absorption Wavelength	Dissolved Organic Concentration
1. Sewage Outfall	3,2,1-1 6,5-2	4,3-4,5		7,6-7,6	3,2-1 4-3,2	275 millimicron	3260 $\frac{mg\ C}{liter}$
2. Kelp Beds	7-3,2 7-6,5	5-6,5	2,1-1 6,5,4-1	7,6-7,6	6-4,3,2	420 324 265-275	2.12
3. Monterey Harbor	4,3-1 5,4-3,2	6-6,5 6,5-6				260	41.3
4. Moss Landing	6,5-1 6,5-4,3 4,3-4,5	5,6-5,4 7-6,5	3,4-1	2,3,4-6	1-1	432 330 274 245	2.42
5. Salinas River	7,6-2 5-5,4	6-6,5	1,2,3-1			255-265	1.49
6. Rip Current	6-3		1,2,3-1				1.31
7. Midwater (100m)	6,5-1 5-3,2 7-3,2	6,5-6		7-7,6	2,1-1	271-281	1.07
8. Midwater (100m)	6,5-1,2 4-5,4	7-4	4,3-1	4-6		320 275 245	1.42
9. Deep Water (1000m)	1,2-1 6-3,2	1-1 6-6,7 7-3,2			6,5-1	275-285	0.56
10. Deep Water (500m)	6-3,2	7-5,4 6,5-6,5	4,3-1		1-1	346-356 262-272	0.93

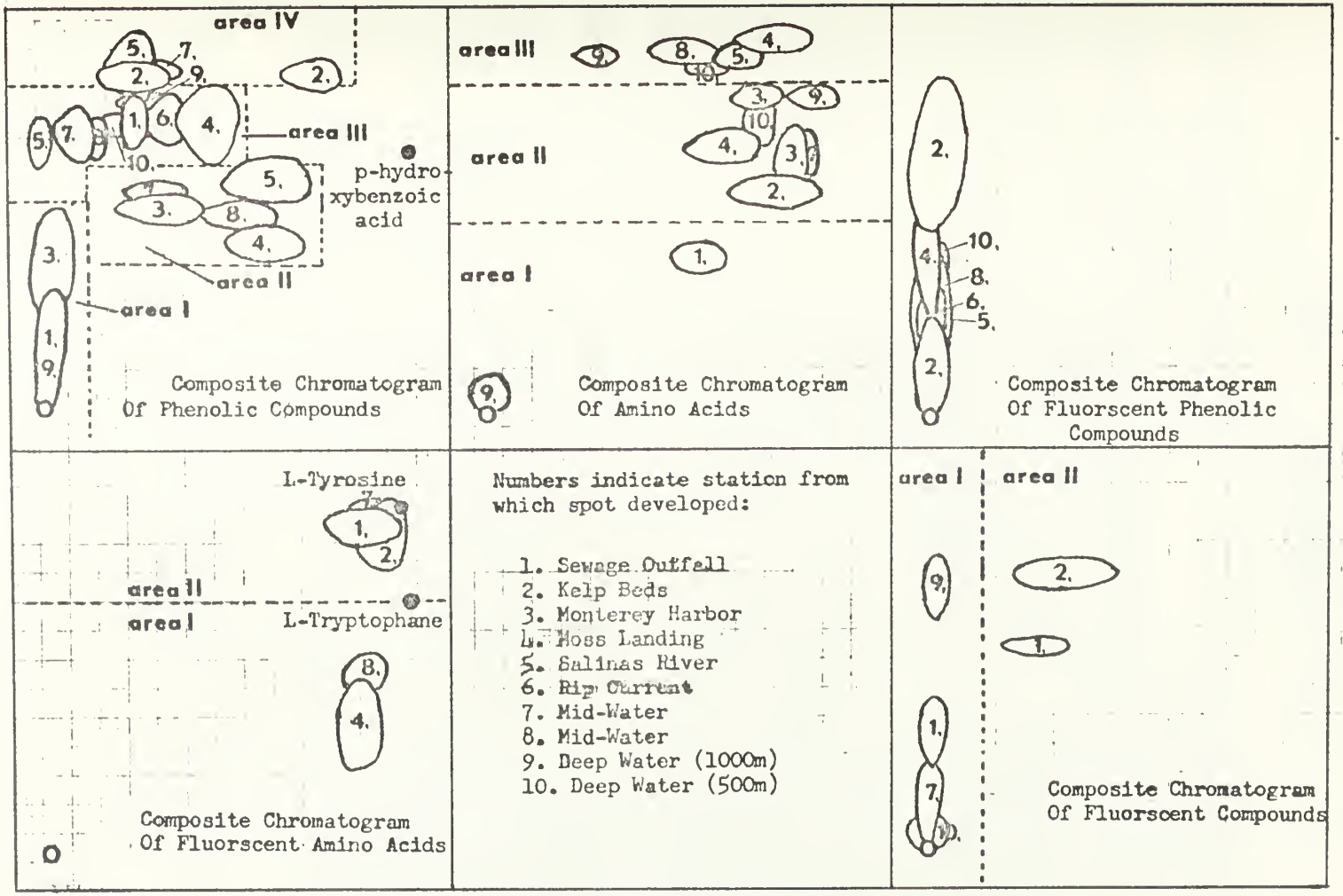
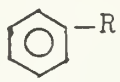
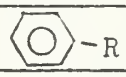
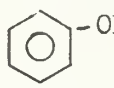


Fig.44. Composite two dimensional paper chromatogram to show positions of the organic functional groups from the different sources.

TABLE V: EXPERIMENTAL RESULTS:
UV-ABSORBANCE

UV-Absorbance (millimicrons)	Sample	Possible Characteristic Organic Groups
245	4-Moss Landing 8-Mid-Water	($>C=C-CO_2H$) ($=N_2$) ($C_6H_5-COCH_3$)
260	3-Monterey Harbor	(R)
274	4-Moss Landing 1-Sewage Outfall 8-Mid-Water L-Tyrosine	($-NO_2$) ($-ONO_2$) 
280	L-Tyrosine	($O=\dot{C}-\dot{C}=O$) ($>C=O$)
320	8-Mid-Water	($O=\dot{C}-\dot{C}=O$)
324	2-Kelp Beds	($O=\dot{C}-\dot{C}=O$)
330	4-Moss Landing	($-N=N-$)
420	2-Kelp Beds	($C=\dot{C}-CHO$)
432	4-Moss Landing	($-N=N-$)
255-265	5-Salinas River	
264-274	2-Kelp Beds 10-Deep Water	 ($-ONO_2$) ($-NO_2$)
274-284	7-Mid-Water 9-Deep Water Procedure I Procedure II	($>C=O$) ($-NO_2$)
346-356	10-Deep Water	($-ONO$)

IV. DISCUSSION

A. SEA WATER SAMPLING

The general hypothesis around which work described in this thesis was carried out, was the suggestion by Sieburth (1968) that natural waters may be characterized on paper chromatograms by their acidic nature, especially phenolic, amino, and fluorescent acids. Chromatograms and UV-spectrum of water samples were taken from potential organic sources in the Monterey Bay area, sewage outfall, kelp bed, harbor area, river mouth, rip current and industrial discharge. Initially, it was presumed that deep water samples would be a good reference station and perhaps represent the refractory end product of the chemical processes undergone by the inshore and surface organic materials. The mid-water samples were obtained in order to attempt to correlate between the sources and the refractory end product. The results partly bear this out, but deep offshore samples do appear to contain compounds typical of inshore and surface sources. The following discussion is a more step-wise account of the techniques and results. Unfortunately, the method of sampling was not the best. An all glass large volume batch sampler would have been far superior to the closely packed string of Nansen bottles which were used for offshore samples.

B. FILTRATION AND CENTRIFUGATION

The separation of particulate and dissolved material by centrifugation and gentle filtration was a most important step. Many experi-

menters in the past have used no or inferior methods or neglected this step completely and, in so doing, they have broadened their research from just dissolved organics to total organic concentrations. It is sometimes argued that there is 100 times more dissolved organic material than particulate and that the latter can be ignored, but since there is not a homogenous distribution of particulates, this is a shaky argument. It is also probably true that the use of vacuum filtration which will rupture cells and the use of membrane filters which leach organics has invalidated the results of researchers who did not take these precautions.

The series of decreasing pore size glass filters provided a stepwise separation of particulate matter and greatly minimized the rate at which any one filter became clogged. In fact, only the 0.45 micron filter required changing and this was infrequent. The inverse flow of the sample up through the filters had two major advantages: 1) gravity pulled the separated particulate away from the filter surface; and 2) living "particulate" by nature concentrated themselves in the low points of the system. These advantages, in addition to the principle of a gentle positive hydraulic head, provided the optimum filtration system.

Experiment I determined the optimum height to be about 2.15 feet. This provided a flow gentle enough not to rupture cells but fast enough so that the sample was not altered because of time dependent processes. As would be expected, the final filter which was the most sensitive to changes in height (Fig. 19). The other filters could have withstood a stronger head, but the exponential increase in broken cell count of the final filter eliminated this possibility.

The broken cell count difference in the low point reservoirs before each filter and past-filter filtrate indicated that for each fritted glass filter, the most damage to cells occurred when the cells passed through the narrow glass channels. Separate experiments on each of these filters confirms this theory. As a result of the suggestion of Dr. E. C. Haderlie, Naval Postgraduate School, a second separation method was investigated involving the use of centrifugation, which has been used in the past by marine biologists to isolate plankton which were presumed to be undamaged. The only centrifuge available was an obsolete three speed model with a medium volume capacity. Experiment II was conducted in order to investigate the effects of centrifugation of suspended cells. The optimum centrifuge time for each centrifuge speed was selected as the time when the suspended cells and broken cells were both at a minimum. From Fig. 15,16 and 17, it was observed that, with an increase in centrifuge time, the suspended cells exponentially decreased, but the broken cells exponentially increased. The optimum time was chosen at the point where the two curves crossed.

Fig. 18 represents the comparison of the different rates and their optimum times. The three systems were equal in their ability to isolate the suspended cells, but the use of 600 RPM for 14 min. produced the least destruction of cells. Thus, for the No. 7342 Precision Scientific Co. centrifuge used, 600 RPM for 14 min. was the optimum rate and time. This system did not isolate all the particulates and, hence, a final filtration was necessary to ensure removal of all particulate matter. Fig. 19 graphically depicts the change in flow rates with different filter systems using tap water adjusted to $35^{o}/_{oo}$ NaCl. The experiment showed that when clogging was not a major factor, the

flow rate could be 1.2-1.3 times faster if only the final filter was used. The obvious conclusion is to design a large volume gentle centrifuge which can make a primary separation sufficient to go directly to the final 0.45 micron filter.

Experiment II was a crude comparison of the two separation procedures. The results from this chemical comparison (Table III) indicate that essentially the centrifugation or filtration systems are equally exclusive for the process time. Chromatographic and UV-absorption patterns were identical within experimental ranges. Dissolved organic concentrations were also equivalent considering the method used, but the difference in process times indicate that the centrifugation method could be 1/4 to 1/3 faster.

The results could be challenged on the basis of the probable existence of a non-homogenous plankton solution used in the different experiments. However, experimental error was minimized by repetition for consistency, reversal of experimental steps, and fresh plankton tows for each experiment.

These experimental results are probably high due to excessive cell breakage because of the high concentrations used which produced additional cell damage due to an increase in the probability of collision and rupture. In the filtration procedure, not only did the cells risk damage in coming in contact with glass filters, but also in competition for available space. Excessive cell breakage due to high concentration would probably be even greater for the centrifugation procedure, since the added particulate mass would be very destructive. Natural concentrations in a large volume centrifuge would represent a much less destructive system.

Furthermore, the particulate organic was only 1-10% of the total organic. If the centrifuge-filter system only removed 90% of the particulate, the experimental error would be between 0.1-1.0%. Such small experimental error would not be significant in the overall results and probably would not be detected by the analysing methods.

C. ADSORBENT COLUMNS

The nylon absorbent column is a potentially valuable tool to chemical oceanography. Nylon can desalt large volumes of sea water while selectively concentrating the phenolic and acidic dissolved organic fraction. It may also be possible to separate the eluant from these columns into different chemical fractions. In the past, there have been few satisfactory methods for concentration and isolation of dissolved organics. The best method hitherto developed was probably the charcoal absorption technique of Braus, Middleton, and Walton (1951) who recovered and estimated 57% of organic exudates. Significantly, the nylon column recovered an average 85% of the phenolic and amino acid standards used. We cannot expect this high recovery to be consistent for all possibly absorbed molecules. The discoloration of the nylon during sample processing indicates that some absorbed compounds did not elute. These yellow pigments were probably polyphenols too large to be removed from the column (Sieburth, 1968).

Hydrogen bonding is believed to be the mechanism by which the nylon adsorbed certain compounds. Hydrogen is peculiar because it is a small atom, but can induce large positive dipoles when bonded on another atom. The hydrogen atom is so strongly positive when bonded to an electronegative atom, it can attract a second electronegative

atom, polarizing its electron atmosphere and giving rise to a strong attractive force of the van der Waals type but much stronger. The relatively great strength of the attractive forces is probably due to the fact that the hydrogen atom possesses no screening electrons. The small size of the hydrogen atom allows the second electronegative atom to approach close enough so that the electrostatic interaction is maximized, but prevents more than two atoms from being linked by the so-called hydrogen bond.

The hydrogen bonds are formed only between quite highly electronegative atoms, for these increase the effective positive charge on the hydrogen atom and account for such attraction between non-metallic atoms. Any factors which increase the electronegative character of the atom originally bearing the hydrogen will increase the tendency toward hydrogen bond formation and the strength of the bonds formed. Hence, phenols form stronger hydrogen bonds than do aliphatic alcohols because resonance with ionic structures enhances the electronegativity of the oxygen in the phenols. Reasonably stable hydrogen bonds are formed only with nitrogen, oxygen, fluorine, and chlorine; thus, for this study, the nitrogen and oxygen bonds are the major interest (Eastman and Rolletson, 1947).

Returning to a discussion of the adsorptive column used in this study, the compounds to be isolated were acidified to insure they were in the acidic form as the phenol on the left hand side of the reaction in Figure 45. When the phenol was placed on the nylon column, hydrogen bonding could occur between the phenols hydrogen and the oxygens and nitrogens of the nylon (see Fig. 46). Figure 47 is the structure suggested by Duursma for humic compounds found in sea water, the acidic

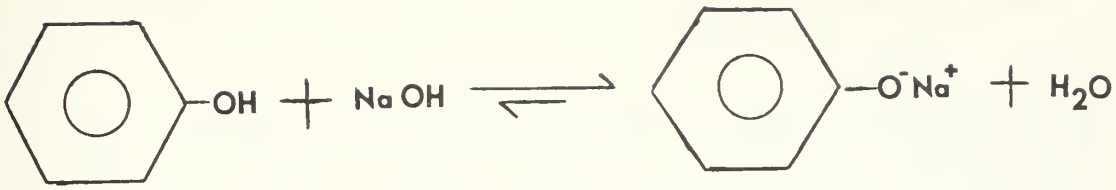


Fig. 45. Phenol Conjugate Acid, Base Reaction

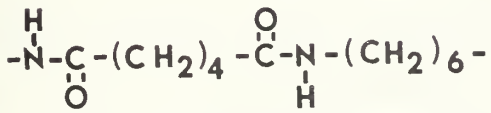


Fig. 46. Nylon Molecular Structure

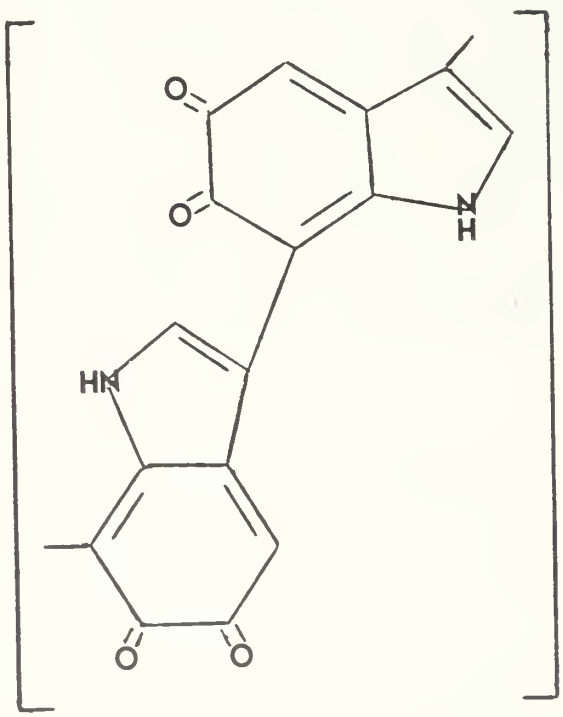


Fig. 47. Duursma's Humic Structure

nitrogens represent locations of possible hydrogen bonding of this type.

When the column is eluted with NaOH, the acidic compounds are simply converted to their conjugate bases (see Fig. 45). They are then washed off the column with water, since the hydrogen bonds have been broken. Phenols, organic acids, amino acids and carbohydrates exhibit this property, but phenols are by far the most common (Cowan and McDonald, 1965). Other common classes of compounds which are not sufficiently acidic to have been adsorbed on the column are aldehydes, ethers, esters, and hydrocarbons.

Basically speaking there are two effects determining which acidic fractions were adsorbed on the column. The first is the inductive effect or the electronegativity effect. As described above, the greater the electronegativity, the stronger the hydrogen bond, and second, the steric effect which concerns size and shape of bonding molecules. Only those compounds which can sterically approach to the nylon bonding sites will be adsorbed. These two facts indicate certain molecules bond more rapidly than others; thus, the better bonding molecules will be found higher on the column. This implies that the nylon could also be used to separate the adsorbed compounds.

D. EXPERIMENTAL CONTROLS

The chromatographic results for distilled water controls (which were passed through the column in the usual way) disclosed characteristic amino and phenolic spots and a lack of fluorescent material. The phenolic spots 6,7-1 and 1-6,7 and amino spots 7-6 and 6-7 appear to have been organic artifacts of the paper or the distilled water and

mixed solvents since they were reproducible when nothing was placed on the chromatogram before development. The absence of distinctive UV-absorbance for distilled water also implies the spots originated in the chromatographic procedure. A third phenolic spot 7,6,5-7 appeared to come from the column since it did not occur on the "chromatographic blank" (developed with distilled water which was not passed through the column). It could have owed its origin to organic contamination in the column system or to the associated salt produced from the neutralization of sodium hydroxide with hydrochloric acid. The UV-absorbance which was characteristic of an inorganic salt infers the latter theory, as did Sieburth (1968) when he found similar spots on his paper chromatogram.

E. ANALYSIS OF SEA WATER STATION DATA

Paper chromatography was chosen over thin layer because it provided an efficient separation without difficulty. Paper chromatography is particularly suitable for samples in the microgram region, and for mixtures of substances that are closely related chemically (Pecsok and Shields, 1968), both of which are true for this experimentation. Paper chromatography solved problems earlier investigators incurred in the failure of their preparations to separate due to insolubility in organic solvents. The use of wet spots, thick paper, and water as the first solvent help overcome these difficulties (Sieburth, 1968). The color reaction of amino groups with ninhydrin (triketohydrindene hydrate), still remains the most important procedure for the detection of amino acids on chromatograms. Unfortunately, the mechanism of the ninhydrin reaction has not yet been satisfactorily explained in all its

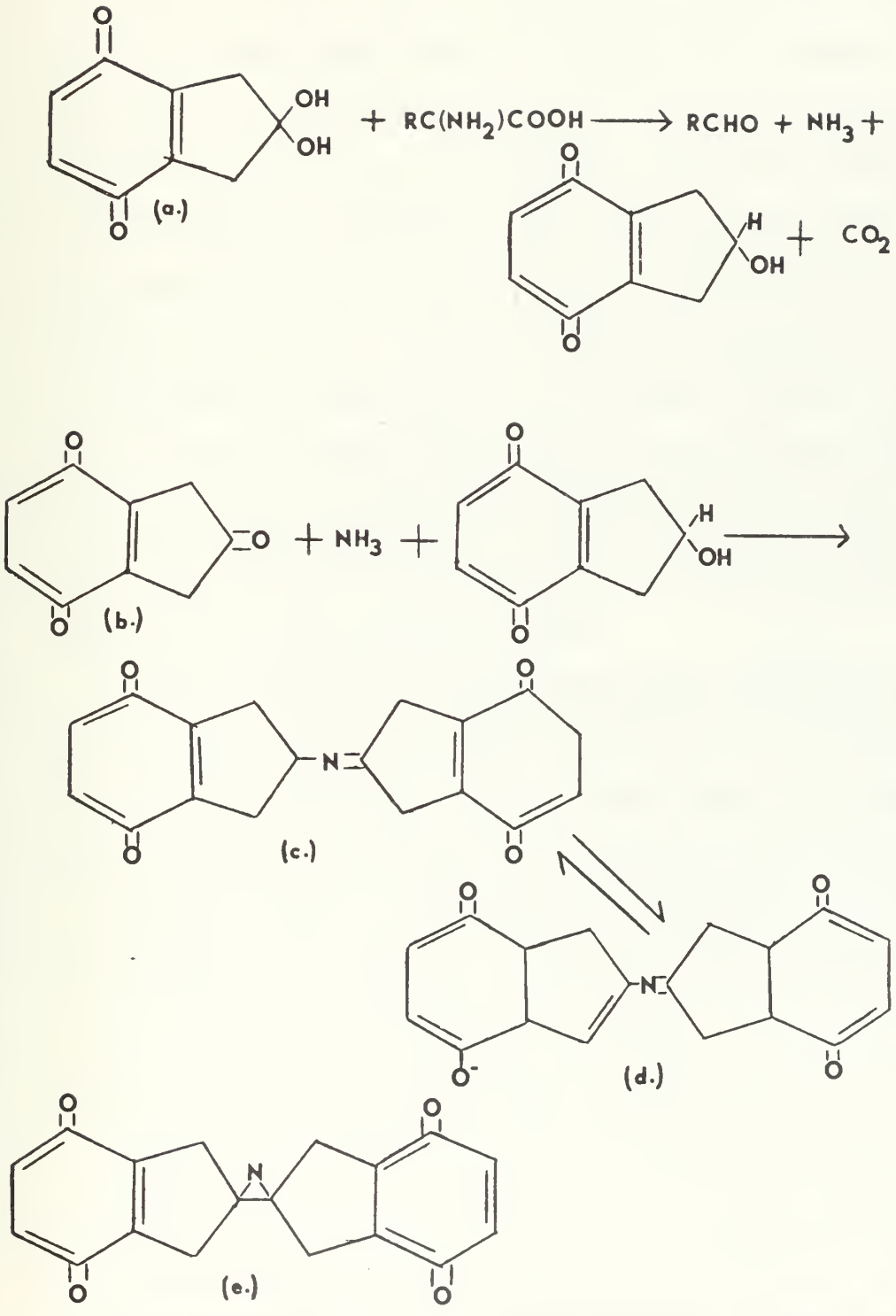


Fig. 48. Ninhydrin Reaction with Amino Acids.

details. However, the main reaction is the Strecker degradation of amino acids by ninhydrin (see Fig. 48),(a) with the formation of carbon dioxide, ammonia, and an aldehyde which is one carbon atom less than the original amino acid. It is assumed that ninhydrin is simultaneously reduced to diketohydrindol (b) which forms diketohylidene-diketohydrindamine (c) or its anion (d) with ammonia liberated and a further molecule of ninhydrin. Meyer (1957) favored formula (e) or its anionic form for the purple product. The dye (c,d,e) is also formed by the action of reducing agents and ammonia on ninhydrin even in the absence of amino groups. It is obvious that the color obtained by the reaction of amino acids on paper is not the only reaction which takes place, since different amino acids give diverse colors and various products must, therefore, be formed, but Figure 48 represents the major reaction (Hais and Macek, 1963).

The color reaction of phenols with ferric chloride is an important method for the detection of phenolic compounds on chromatograms. The reaction is a substitution of the phenol's oxygen for the chloride in the irons inner coordination sphere producing a blue pigment.

Figures 21 and 22 show the different distributions of compounds for the different water samples. The characteristic patterns for each station form a map of its phenolic, amino, and fluorescent compounds and, as can be seen, there are unique separations for each station.

The analysis of the results from the chromatography are augmented by UV-absorbance data. Energy absorbed in the ultra-violet region (200-450 millimicrons) produces electronic transitions to higher energy levels within the molecule. The absorption is dependent upon the probability of occurrence of interaction between energy and electron

system, which would raise the ground energy level to an excited state, and the polarity of the excited state. Ultra-violet spectra reveal fewer structural features than other instrumental methods such as infrared; whereas, many compounds are transparent in the near ultra violet (Silverstein and Basslar, 1968). Since sea water samples are not pure solutions but complex mixtures of compounds, the partial selectivity of UV-analysis helps to eliminate some of the possibilities in the characterization of the dissolved organic material of these water masses.

The dissolved organic content provided some insight into the role of the different sample stations as organic sources or sinks. Where the paper chromatography and UV-spectrometry characterized the type and range of organics, the organic concentrations determined the relative importance of the different sources.

A detailed analysis of the sewage outfall data from Table IV will be used to show how the table should be read and the general conclusions which can be reached. The sewage outfall (Pacific Grove, Calif.) was a major source of dissolved organic material as would be expected. Excessively high concentration compared to the other stations is due to the fact that the sample was obtained at the outfall, before the organics were mixed into the sea. This sewage water was 100 times more concentrated in dissolved organics than any other station. Based on the reaction and position of spots on the chromatogram (see Table IV and Figure 21) the station had phenolic compounds one (P 3,2,1-1) of which was slightly soluble in water and insoluble in the organic solvent, and the other (P 6,5-2) which was soluble in water and slightly soluble in the organic solvent. Two amino compounds were present, one

amino compound (A 4,3-5,4) was moderately soluble in both water and the organic solvent; the other was a highly, water and organic solvent, soluble fluorescent amino compound (Pf 7,6-7,6). Finally, there were two fluorescent compounds both of which were slightly soluble in water and insoluble in the organic solvent. The sewage also showed a lack of any fluorescent phenolic compounds. The UV-spectrum of the sewage outfall showed one major peak at 275 millimicrons. This absorption spectra is characteristic of Kalle's (1960) "Gelbstoffe." The relative solubility of each chromatographic spot can be determined in this general way.

This accumulation of data allows the chemical description of each station relative to paper chromatography, UV-spectrometery, and dissolved organic content.

The sewage outfall is an area of extreme organic concentration, for a variety of compounds. Individual compounds share a similarity with compounds from other sources, e.g. the kelp beds, Monterey Harbor, and Moss Landing. One may speculate that the sewage outfall is an organic source for mid-water and deep water masses of Monterey Bay. The pollution being added to the ocean through this source should be studied in more detail regarding the chemical nature, concentration and distribution of compounds.

The concentrations and variety of organic compounds present in the kelp beds indicate a reasonably rich natural source of biological material. According to Sieburth (1969), algal compounds contribute to the Gelbstoffe. The compounds are similar to compounds in the sewage outfall and Moss Landing suggesting that they are products of metabolism. Relationships with other stations implies that kelp may be an important

source of dissolved organics for mid-water and deep water masses in Monterey Bay.

Monterey Harbor is a relatively stagnant enclosure of relatively high organic concentrations. The lack of fluorescent compounds was unique for this station. The chemical similarity with the sewage outfall would be expected in an area of boat discharge. The organic concentration and the limited functional groups present indicate a definite lack of circulation with the natural bay water. The harbor does not appear to be a major source for mid and deep water, and is not characteristic of "Gelbstoffe."

Moss Landing may be a major source of organics for the bay, as indicated by its relatively high organic concentration and wide variety of compounds. The paper chromatography and UV-spectra indicate many functional groups in low concentrations, and as would be expected, "Gelbstoffe" is definitely present. The concentration and distribution of compounds and similarity to mid and deep water chemical patterns indicate good circulation in the Moss Landing area. The high organics may directly or indirectly be related to local industrial discharge from the Kaiser Plant, or increased biological activity due to the discharge of hot water into the bay at this point from the Pacific Gas and Electric power plant.

The Salinas River source appeared characteristically different from the other stations. The dissolved organics in the river runoff were easily distinguishable from other sources and appear to be traceable into mid and deep water.

The rip current was surprisingly low regarding organics, and contained a very limited range of compounds. The compounds showed some

similarity with the Salinas River. It was originally hypothesized that the rip current might pump accumulated organics away from the coast, but no evidence of this was found, or possibly this station was not really in a rip current.

Mid-water station 7 was in the middle of the bay and suggested origins especially from the sewage outfall and kelp beds. The organics were predominately phenolic in nature with the presence of "Gelbstoffe" clear.

Mid-water station 8 was in the Monterey Canyon closer to Moss Landing which may explain an increased organic concentration. The major sources for this water mass are most likely Moss Landing and the Salinas River. This mid-water contained "Gelbstoffe" and a variety of other compounds. The difference between station 7 and 8 indicates the samples were taken from different water masses.

The deep water station 10 appears to be the accumulation of organic sources in the bay especially the kelp beds, sewage outfall and Moss Landing. Since it contains similarities of both mid-water stations, there appears to be mixing of water masses at this depth. There are also indications of "Gelbstoffe" being present even in these deep waters.

Deep water station 9, while it contains some "Gelbstoffe," is chemically different from deep water station 10 and the other bay sources, indicating either a chemical alteration or that this water mass may be a bottom current, unrelated to Monterey Bay circulation, bringing organics up the coast.

F. ORGANIC STANDARDS

The chromatographic and spectrometric data indicates the probable existence of L-tyrosine and the possible absence of L-tryptophan and p-hydroxybenzoic acid. Figure 23 shows the relative positions of the three standards to the other chromatographic spots. L-tyrosine is a possible constituent of the dissolved organics from the sewage outfall kelp beds, and the mid-water samples. These are all areas with a history of biological activity in which the presence of L-tyrosine would not be unreasonable. The UV-spectrum also suggests the possible presence of L-tyrosine at these stations. The lack of chromatographic and spectrometric correspondence between the stations and L-tryptophan and p-hydroxybenzoic acid experimentally implies their absence in the sampled waters.

V. SUGGESTIONS FOR FURTHER STUDY

A significant portion of the work described in this thesis has been to develop and refine a system for the routine concentration and desalting of large volumes of sea water. The discoveries made during research have indicated a more efficient system could be built. The new system would vary from the old in four areas. First, a large volume glass sampler should be obtained so that volumes of sea water up to 20 liters can be taken without contamination, inconvenience, and experimental error. The glass sampler should be washed as described except that organic-free distilled water prepared by irradiation under UV light should be used as a final rinse. Diehl (1971) described the best batch samplers in use. With this new sampling method, the stations reported here should be sampled repeatedly in order to confirm the hypothesis, and to determine the time dependence of the dissolved organic concentrations.

Second, a large volume centrifuge should be built so that the large volume samples can be precentrifuged gently before filtration. The centrifuge should be designed to handle up to two 20 liter samples including the glass samplers referred to by Diehl (1971). The apparatus should be driven by a variable speed motor so that the optimum centrifuge rates and times can be experimentally determined as in the filtration-centrifugation section of this thesis. The same rates and times that were determined in this thesis might not be the best for larger scale work.

The third improvement would be the use of a large surface area

0.45 micron glass filter. The small diameter of the 4cm filter used in this thesis was one reason for the slowness of the filtration process.

The final difference in the new system would be the use of the nylon column as a fractionating chromatograph. The theory behind the chemistry of the column indicates molecules being absorbed at different rates which implies that the column could be used to separate compounds for analysis. The optimum method would probably be a step-wise column system employing as many as five different nylon columns. The sea water sample would be placed on the first column and fractionated into five, ten milliliter fractions, and each of these fractions placed on the next column and fractionated again. Thus, the nylon column not only would concentrate the sample but also desalts and fractionates it. These advantages make it an extremely powerful tool in the investigation of trace organics.

After the organics were isolated and fractionated, analysis would be much easier and new methods of analysis would be possible since the complex mixture of compounds would be simplified. Fluorescence spectrometry could become very important since it gives characteristic emission curves with small concentrations. Paper chromatography and UV-spectrometry should also be used to correlate data previously collected. The use of infrared, mass, nuclear magnetic resonance, and electron spin resonance could then become powerful tools for identification of isolated compounds.

The ability of these nylon columns to isolate phenolic compounds may be very useful in the study of phenolic pollutants. Such methods are of great importance to the government which is looking for new ways of studying pollution and ways of preventing it.

VI. SUMMARY

1. The separation of the dissolved and particulate organic material was best achieved by a combination of gentle centrifugation, and inverse flow filtration using a gentle positive hydraulic head.

2. Nylon absorption columns were used to concentrate and desalt humic acids, especially phenolic and amino acids, from sea water samples.

3. Paper chromatography and UV-spectrometry were used to characterize organic sources and water masses according to their patterns of dissolved organic material.

4. Using these characteristic "mappings," i.e. the paper chromatograms, it appears possible to trace movements of dissolved organic compounds from sources to mid-water and on into deep water.

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Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Naval Postgraduate School
Monterey, California 93940

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
Characterization of Gelbstoffe in Monterey Bay
by Nylon Adsorption, UV, and Paper Chromatography

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Master's Thesis; March 1972

5. AUTHOR(S) (First name, middle initial, last name)
Laird William Lewis, Jr.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
March 1972	98	36

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO.	
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10. DISTRIBUTION STATEMENT
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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
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13. ABSTRACT

The dissolved organic components of marine waters were studied using an inverse flow glass filter system to separate the particulate and dissolved constituents. Cell rupture was prevented by the use of a gentle positive hydraulic head. Concentrations of humic acids were obtained by a nylon adsorption column which also desalted the sample. The process provided concentrations up to 10,000 times. Paper chromatography and ultra-violet spectrometry were used to characterize the dissolved humic content of water from Monterey Bay and offshore ocean stations. The correlations between sources indicated the relative importance of organic acids, amino acids, phenols, and carbohydrates in sewage outfalls, kelpbeds, harbor areas, river mouths, rip currents and industrial discharges.

DD FORM 1 NOV 65 1473 (PAGE 1)
S/N 0101-807-6811

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dissolved organics						
nylon adsorption						
humic substances						
phenols						
seawater						
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